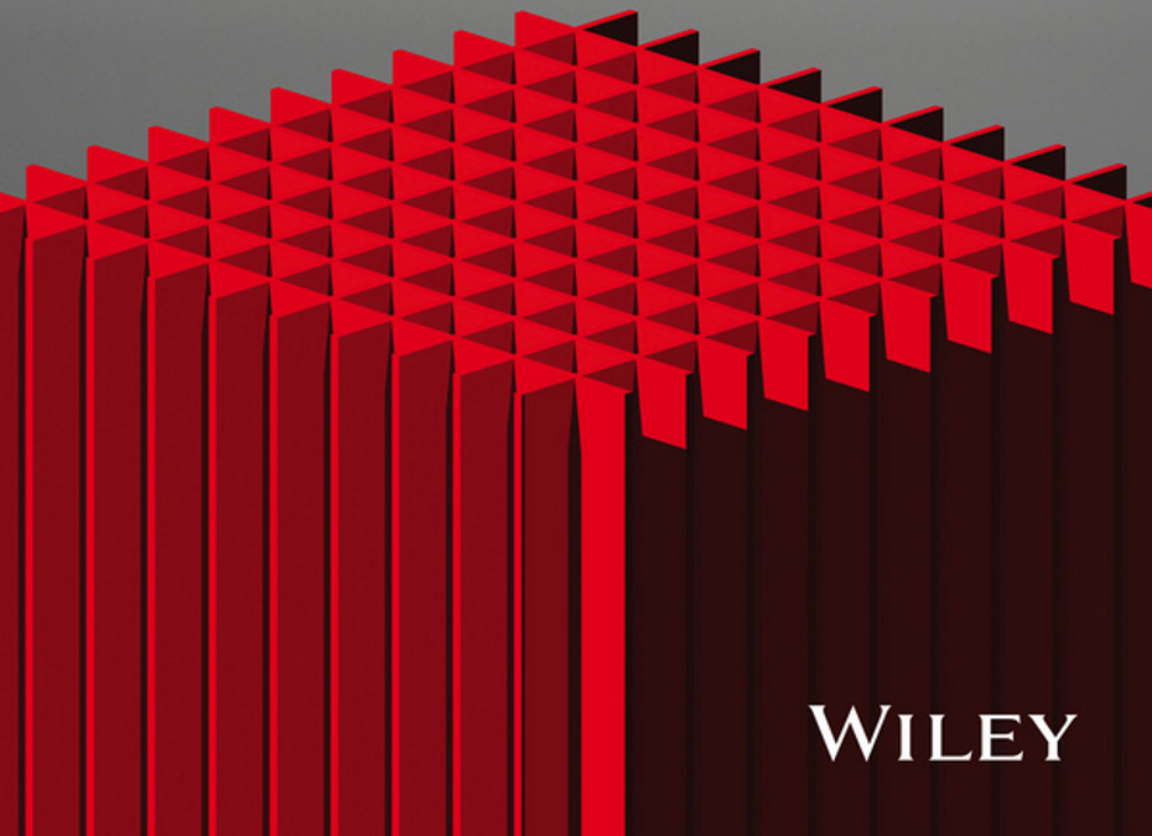
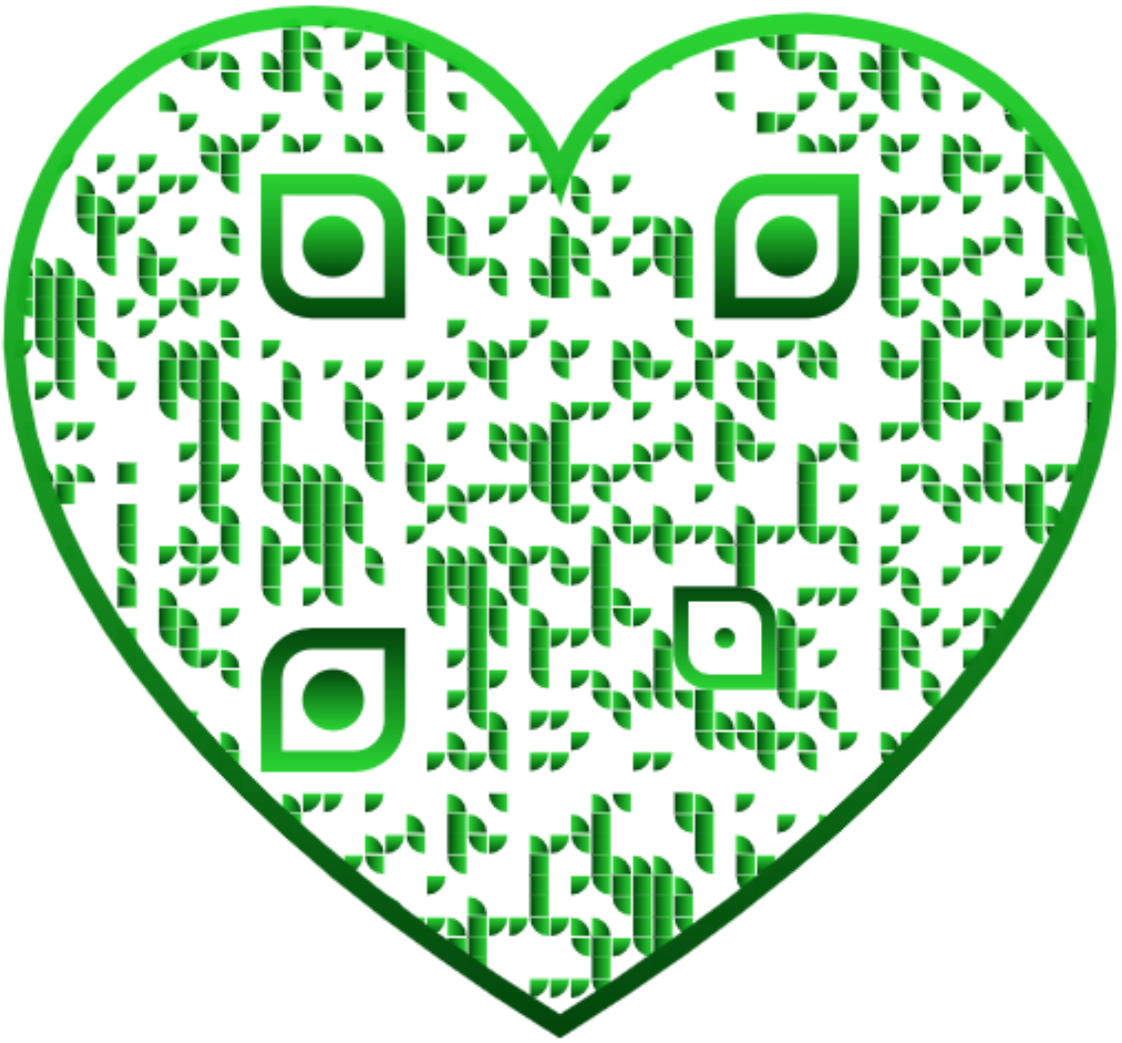
ICH Quality Guidelines

An Implementation Guide

Edited by Andrew Teasdale, David Elder, Raymond W. Nims



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Edited by

Andrew Teasdale
AstraZeneca, London, UK

David Elder
Consultant (Former GSK), Hertford, Hertfordshire, UK

Raymond W. Nims
RMC Pharmaceutical Solutions, Inc., Longmont, CO, USA

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List of Contributors

Morten Allesø
Chemical and Pharmaceutical Research
H. Lundbeck A/S
Valby
Denmark

Joel P. Bercu
Gilead Sciences, Inc.
Foster City, CA
USA

Phillip Borman
GSK
Ware
UK

Mette C. Bryder
Chemical and Pharmaceutical Research
H. Lundbeck A/S
Valby
Denmark

Jeanine L. Bussiere
Amgen Inc.
Thousand Oaks, CA
USA

Patricia W. Cash
MedImmune
Gaithersburg, MD
USA

David Clapham
David Clapham, Independent Pharmaceutical Consultant
Hertfordshire
UK

John Connelly
ApoPharma Incorporated
Toronto, Ontario
Canada

John G. Davies
MedImmune
Gaithersburg, MD
USA

David Elder
David P Elder Consultancy
Hertford
UK

Daniel Galbraith
BioOursource Ltd.
Glasgow
UK

Di Gao
AstraZeneca
Frederick, MD
USA

Richard Harris
AstraZeneca
Frederick, MD
USA

James Harvey
GSK
Ware
UK

Per Holm
Chemical and Pharmaceutical Research
H. Lundbeck A/S
Valby
Denmark

René Holm
Chemical and Pharmaceutical Research
H. Lundbeck A/S
Valby
Denmark

Yoen Joo Kim
MedImmune
Gaithersburg, MD
USA

Kim Li
Amgen Inc.
Thousand Oaks, CA
USA

Zhong Liu
Merck & Co. Inc.
Kenilworth, NJ
USA
and
Currently at: Adello Biologics
Piscataway, NJ
USA

Robert McCombie
Genentech Inc.
San Francisco, CA
USA

Raymond Peter Munden
Munden Consultancy
Royston
UK

Gordon Munro
Munro-Elbrook Associates
Welwyn
UK

Raymond W. Nims
RMC Pharmaceutical Solutions, Inc.
Longmont, CO
USA

Ronald Ogilvie
Pfizer
Sandwich
UK

Danny Ooi
Genentech, a Member of the Roche Group
South San Francisco, CA
USA

Mark Plavsic
Lysogene
Cambridge, MA
USA

David Pollard
Merck & Co. Inc.
Kenilworth, NJ
USA
and
Currently at: Amicus Therapeutics
Cranbury, NJ
USA

Qiang Qin
AstraZeneca
Frederick, MD
USA
and
Current Affiliation: GlaxoSmithKline
Rockville, MD
USA

Jinshu Qiu
Amgen Inc.
Thousand Oaks, CA
USA

Anil Raghani
Coherus BioSciences, Inc.
Camarillo, CA
USA

Ramani R. Raghavan
Merck & Co., Inc.
Rahway, NJ
USA

Andy Rignall
AstraZeneca
London
UK

Scott R. Rudge
RMC Pharmaceutical Solutions, Inc.
Longmont, CO
USA

Timothy L. Schofield
Current Affiliation: GlaxoSmithKline
Rockville, MD
USA
and
MedImmune
Gaithersburg, MD
USA

Garry Scrivens
Pfizer
Sandwich
UK

Steven Spanhaak
Janssen Pharmaceutica NV
Beerse
Belgium

Andrew Teasdale
AstraZeneca
Macclesfield
UK

Sarah Thompson
AstraZeneca
Macclesfield
UK

Larry Wigman
Genentech, a Member of the Roche Group
South San Francisco, CA
USA

Jianxin Ye
Currently at: Amicus Therapeutics
Cranbury, NJ
USA
and
Merck & Co. Inc.
Kenilworth, NJ
USA

Roujian Zhang
AstraZeneca
Frederick, MD
USA

An Introduction to ICH Quality Guidelines

Opportunities and Challenges

The International Conference on Harmonisation (ICH) of technical requirements for registration of pharmaceuticals for human use was initiated in April 1990. ICH had the initial objective of coordinating the regulatory activities of the European, Japanese, and the United States bodies (along with the pharmaceutical trade associations from these three regions), to discuss and agree the scientific and technical aspects arising from product registration. This was recently supplemented by the addition of Health Canada and Swissmedic, to the core ICH Steering Committee (SC) [1].

At the initial ICH SC meeting the terms of reference were agreed and it was decided that harmonisation initiatives would be divided into Safety (S), Quality (Q), and Efficacy (E), reflecting the main criteria which underpin the approval and authorization of new medicinal products. It was subsequently realised that several topics were multi-disciplinary (M) in nature.

Thus, ICH's mission was to realize greater harmonization in both the interpretation and application of requirements for new product registration, with the objective of minimizing repetition/duplication of both testing and reporting, which is routinely performed as part of the development of new medicinal products. Harmonizing these differences via the ICH guidelines would help industry reduce development times, save resources and benefit the patient.

It is difficult not to underestimate the benefits of the ICH initiative in general and the ICH Quality guidelines in particular (and those related Multi-Disciplinary guidelines), to the CMC community. Although it is fair to state that not all of the guidelines have been equally successful; it is very clear that the majority have been very successful and there is an ongoing recognition of the need to update and maintain the guidance in line with new developments and technological advances. Furthermore, the desire to extend the benefits of harmonisation beyond the ICH regions through collaborative efforts is to be welcomed and brings us a step closer to global harmonisation of these important principles of medicinal product evaluation. As part of the objective to extend its global outreach, ICH recently welcomed new regulatory members

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from Brazil and South Korea. In addition regulatory authorities from Cuba, Kazakhstan, and South Africa were also agreed as ICH Observers [2].

The success of the ICH guidelines, in many ways has been due to the adoption of overarching principles and a guidance framework describing the main requirements for compliance without being overly prescriptive. Yet while varying levels of detailed information has been included in the different guidelines to facilitate understanding, it has left many seeking further clarification on the practical application of the guidance. The purpose and benefit of this book is that it allows the reader a deeper insight provided through dedicated chapters into the practical aspects of a specific guideline's application.

Each of the chapters seeks to examine the key requirements of the specific guidelines and then considers the challenges both in interpretation and practical implementation. It is this perspective, looking behind the basic framework; and then examining both the intent and practical guidance that I believe will make this text an essential aid to those involved in CMC matters, both from an industry and regulators' perspective.

To achieve the intended goal the Editors have pulled together an unrivalled collation of subject matter experts aligned to each chapter, many involved directly in the derivation of the ICH guidelines themselves.

Dr David Tainsh, Chief Product Quality Officer, GSK

References

1 SwissMedic. ICH Meeting in Minneapolis, USA: SwissMedic and Health Canada Included as New Members, July 9, 2014. https://www.swissmedic.ch/aktuell/00673/02270/index.html?lang=en. Accessed on February 27, 2017.

2 ICH. Press Release Osaka Meeting, November 17, 2016. http://www.ich.org/ichnews/press-releases/view/article/ich-assembly-osaka-japan-november-2016.html. Accessed on April 12, 2017.

1

ICHQ1A(R2) Stability Testing of New Drug Substance and Product and ICHQ1C Stability Testing of New Dosage Forms

Andy Rignall

AstraZeneca, London, UK

1.1 Introduction

A core part of the medicines development process is an understanding of the chemical and physical behavior of the active ingredient and the medicinal product into which it is incorporated under the storage and usage conditions they are likely to encounter. The International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) stability guidance provides a foundation and framework for this endeavor.

Stability testing was one of the first quality, safety, and efficacy topics harmonized across the ICH territories (Europe, USA, Japan, Canada, and Switzerland) in tripartite guidance. The latest revision of ICHQ1A Stability Testing of New Drug Substances and Products was adopted in 2003 [1]. It forms the parent guideline to a suite of associated guidelines providing more details on recommended stability practice. The guideline provides information on storage conditions and duration and testing requirements that should be used to generate the core stability data package in support of product registration in the ICH regions. To encompass the behavior of different drug delivery platforms and their input drug substances, the guideline contains some flexibility in the requirements. Importantly, the guideline also includes an introductory statement recognizing that alternative stability approaches can be used if scientifically justified. A short annex to the parent stability guideline is embodied in ICHQ1C, which addresses the stability requirements for a new dosage form when an applicant develops a new product variant following an original drug substance and drug product application [2].

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As worldwide registration is the goal for many medicinal products, the standardization and simplification of the global supply chain for a new medicine, via harmonized stability and labeling practice, is desirable. While the intent of the guideline is to recommend the data sets required to register new drug substance and products in the three main ICH regions, its content is cited and used much more widely. The ICH guidelines are also referenced in territorial guidance beyond the ICH regions either on a stand-alone basis or in support of local stability guidance. For example, the World Health Organization (WHO) is a long-standing observer of the ICH process, leading to the incorporation of much of the content of the ICH into its own stability guidance [3].

The ICH stability guidance not only is intended for registration purposes but also informs stability practice during development, for example, the storage conditions described in the guidance can provide a framework for the development stability protocols used to underwrite the quality, safety, and efficacy of drug product used in clinical studies.

While the guidance embodies a traditional approach to stability protocols, the principles described in terms of the stability performance requirements for pharmaceutical products have also been translated into targets for predictive stability screening tools. These tools can provide assurance that when formal stability studies to support product registration are performed in accordance with ICH guidance, the likelihood of obtaining unexpected results is reduced.

Some stability testing requirements are linked with specific product platforms and are detailed in other guidance. Examples include instructions relating to studies that justify in-use storage, strategies to demonstrate the suitability of protective secondary packaging, and specific studies to underwrite temperature excursions during storage and transportation.

In the "quality by design" era, where pharmaceutical development practice is guided by science- and risk-based approaches, highlighted in three more recent ICH guidelines on pharmaceutical development [4], risk management [5], and pharmaceutical quality system [6], the focus for stability studies has evolved further to emphasize the importance of generating detailed stability knowledge and understanding. This may include establishing the attributes of the input materials (drug substance and excipient) and any processing parameters that are critical to stability performance. Following identification of the attributes critical to stability, an integrated control strategy should be established to ensure the attributes remain within acceptable limits, thereby assuring that the required stability performance is demonstrated. The use of risk management tools to ensure development activities are focused on the areas that will have the most influence on the control of stability (and therefore quality safety and efficacy) is also a feature.

From a practical perspective, the goal of performing stability testing on products intended for global registration remains challenging, requiring the development of a protocol that will result in a high probability of approval in all

major markets. Regions with their own specific stability requirements can make the development of a truly "global" registration protocol more challenging. For example, the guidance on stability study requirements for the registration of drug products in countries forming the ASEAN region of Southeast Asia recommends a different long-term storage condition compared with the ICH regions [7].

This chapter aims to provide an understanding of the fundamental principles behind stability testing and then demonstrate how the guidance is typically applied during pharmaceutical development.

1.2 The Fundamental Science That Underpins Stability Testing

1.2.1 The Stability Process

Quality, safety, and efficacy must be maintained throughout the shelf life of a medicine, from manufacture to the end of shelf life and when being used by the patient. This can be achieved by developing an understanding of the chemical and physical properties of the product so that it is possible to establish methods to control and monitor the critical parameters and establish the long-term behavior of the drug substance and medicine.

The regulations require an expiry date on drug products or a retest date on active pharmaceutical ingredient [8]. For drug product, the expiry date defines the period within which the drug product is expected to comply with its approved control specification limits when stored under the recommended conditions. Similarly, a retest date is assigned to drug substance. If a drug substance batch is required for drug product manufacture beyond its labeled retest date, it should be retested to confirm continued compliance with specification prior to use. Stability testing provides the means to investigate how a medicine behaves under different environmental conditions and demonstrate that a pharmaceutical product maintains its fitness for use throughout this labeled shelf life. The stability testing of drug products involves evaluating them on storage over time in the container/closure system intended for use in the clinic or the commercial market.

The stability of a pharmaceutical product is the result of a complex interplay between environmental factors (temperature, humidity, availability of oxygen, and exposure to light), and the intrinsic chemical and physical stability of active ingredients and formulation excipients. The conditions under which these ingredients are processed to form the medicine, and the degree of protection provided by any primary and secondary packaging are also influencing factors.

The stability process involves finding out what degradation pathways are available to a new chemical entity, what steps can be taken to assess the extent

of degradation most likely to be encountered under normal storage, and what strategies are available to prevent or limit any observed degradation. Chemical breakdown constitutes a major factor in drug or formulation failure on storage, but physical, biological, and microbiological changes can also be a source of instability.

Chemistry driven changes include changes in product quality or product performance characteristics caused by

- Increase in levels of degradation products with potential impact on safety
- Potency loss associated with chemical breakdown/reaction of active ingredient with potential impact on efficacy
- Change in visual, taste, or odor caused by increased levels of degradation, with potential impact on overall product acceptability

The extent of chemical breakdown does not need to be significant for potential problems to occur, for example, formation of low levels of a breakdown product that gives rise to specific safety concerns or small amounts of a highly colored degradation product affecting visual appearance.

1.2.2 Factors Affecting Stability

Demonstrating stability knowledge and appropriate control involves developing an understanding of the factors that can affect the stability of a medicine and confirming that appropriate controls are in place to assure quality, safety, and efficacy throughout the labeled shelf life. These factors include

- The intrinsic stability of the active pharmaceutical ingredient(s)
- Input excipient properties and how they affect the stability of the API
- The unit operations associated with the manufacturing process
- Environmental factors (external, internal, and microenvironment)
- The materials and functionality associated with any packaging system

Further factors affecting product stability are outlined in Figure 1.1

1.2.2.1 Intrinsic Stability of the Active Pharmaceutical Ingredient

Pharmaceuticals are often developed as salts of organic acids or bases in order to achieve the desired physicochemical properties. Ironically often the functionality that imparts the desired efficacy in a medicine may also make the molecule less stable. Pharmaceuticals are generally composed of carbon skeletons with additional functional groups. Electron distribution, bond polarity, and steric factors associated with the carbon skeleton can all affect stability. Factors affecting the electron distribution or electron density, in a drug molecule, can greatly affect its susceptibility to degradation. For example, double bonds alternated across a structure can impart stabilizing conjugation via delocalized electron density. The conjugation can make a molecule more rigid

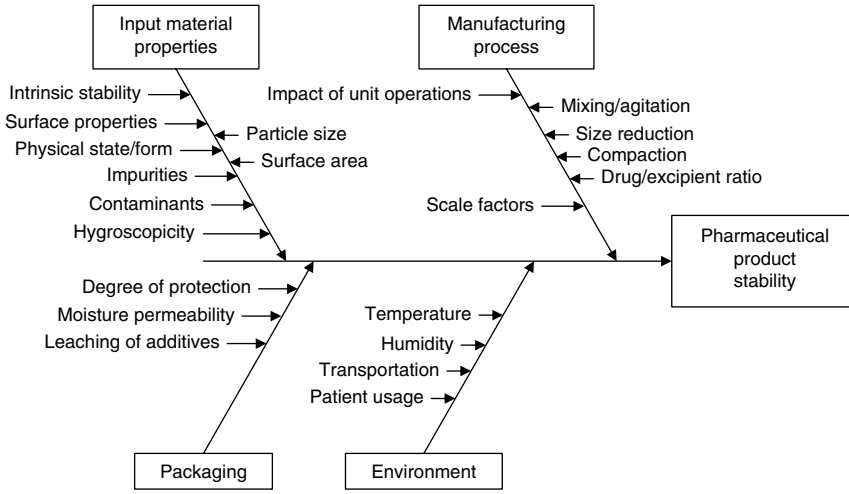


Figure 1.1 Factors potentially affecting product stability.

and also enhance the absorption of UV light. The delocalized electrons can act as a conduit for the transmission of electron density or full negative or positive charge, and this resonance can stabilize charged species making them more likely to form. Inductive effects can result in bond polarization, a movement of electron density across the bond depending on the electronegative or electropositive nature of substituents. Substituent groups can profoundly affect the reactivity, and therefore stability, of pharmaceuticals. Neighboring substituents may interrupt conjugation inhibiting electron delocalization and the potential for resonance stabilization. More often neighboring substituents, especially the bulky ones, prevent a reagent getting to the reaction site, particularly true for nucleophilic substitution (S_N2) reactions. Similarly, the presence of a bulky counterion such as chloride can protect the salt-forming center of the drug substance from oxidative attack. These are all examples of steric hindrance.

Knowledge of the shape and molecular arrangement in tandem with the knowledge of how functional groups react enables theoretical prediction on how a new chemical entity might behave under given physical and chemical conditions [9].

Knowledge of the environment within the formulation allows prediction of those conditions that may initiate or catalyze the reactions causing breakdown.

Therefore, developmental stability programs should be designed to demonstrate how robust a product is to environmental conditions of heat, humidity, and light. These programs are often more extreme than the default ICH storage conditions as there is a desire to accelerate the degradation to

allow meaningful decisions to be made in shorter time periods. Screening studies to investigate chemical reactions with extraneous species such as process impurities or formulation or packaging additives should also be considered as well as studies to assess the potential for physical changes to occur on storage.

1.2.2.1.1 Degradation Reaction Pathways

Chemical Breakdown There are four main reaction mechanisms, all of which may occur as part of a degradative process: substitution, addition, elimination, and rearrangement. Degradation reactions can be broadly categorized as hydrolysis, oxidation, photolysis, and isomerization or rearrangement. In addition, specific interactions between the active molecule and functional groups on excipients, process-related impurities, or extraneous contaminants may also result in additional degradation reaction pathways.

Hydrolysis Due to the ubiquity of water as a potential reactant, the most prevalent reaction mechanism associated with degradation reactions is hydrolysis. Derivatives of carboxylic acids such as esters, amides, imides, lactones, lactams, and acid chlorides are particularly susceptible [10]. It is difficult to completely eliminate water from solid drug products, and it is also the most commonly used solvent for parenteral products. The general reaction for carboxylic acid derivatives is shown:

$$\mathrm{RCOOR' + H_2O \rightarrow RCOOH + R'OH}$$

Water acts as a nucleophile attacking electron-deficient or electropositive sites within drug molecules. The acyl derivatives ester, amide, and imide are most prone to attack, and acid or base can catalyze the reaction.

Alkaline hydrolysis involves the initial nucleophilic attack of a hydroxide ion on the electropositive center and then, for example, in the case of an ester, elimination of the alkoxy substituent to form an acid and alcohol.

In acid-catalyzed hydrolysis of esters, the carbonyl oxygen is protonated and then water nucleophilically attacks the electron-deficient carbon center. Aromatic esters are more reactive compared with aliphatic.

The products are the same via either mechanism. Heat/light and local pH can also catalyze hydrolysis.

Although this is a second-order reaction, if water is present in a large excess, it will exhibit pseudo-first-order kinetics.

A similar situation occurs with solvents that can act as nucleophiles and this is termed solvolysis. The polarity (or dipole moment) of the solvent is an important factor in such reactions.

Protecting an active ingredient that is prone to hydrolytic degradation naturally involves limiting the presence of unbound water, which would be available for reaction. Using a suitable moisture barrier with low water permeability in

the primary and/or secondary pack will reduce the amount of moisture being transported from the external environment inside the pack. Employing a desiccant such as silica gel, using excipients with low moisture content or excipients that can adsorb unbound water such as colloidal silicon dioxide, will also help to maintain low humidity levels inside the protective pack. As extremes of pH can cause hydrolytic breakdown, the use of buffers (more often in solution dosage forms but there are solid state buffer systems in use as well) can stabilize a system.

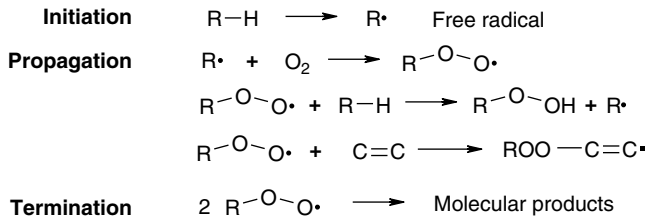
Oxidation Due to its presence in the atmosphere, oxygen represents another readily available reactant for potential degradation mechanisms. Where the reaction occurs with aerial oxygen, this is often called "autoxidation." The oxidation mechanism also includes the addition of an electronegative atom, such as oxygen, or a radical or the loss of an electropositive atom, such as hydrogen, a radical or an electron [11]. Aldehydes, amines, ethers, phenols, thiols, thioethers, and unsaturated fat/oil functionalities are particularly prone to oxidation. The mechanism and resulting degradation product profile can be complex, and it is often associated with discoloration.

General reaction: $R-R+O_2 \rightarrow R-OO$

Oxidation is probably the second most common degradation mechanism.

Oxidation of pharmaceuticals often occurs by direct reaction with atmospheric oxygen under ambient conditions. Usually this is a free radical reaction resulting initially in the formation of hydroperoxides that may react further. This is called auto-oxidation.

The free radical mechanisms can be complex involving initiation, propagation, and termination steps, and many auto-oxidation reactions can be catalyzed by trace amounts of metal ions or hydroperoxides present as impurities. For example:



A free radical is a highly reactive atom or molecule with an unpaired electron. Formation of a free radical, in an initiation step leading to further reaction, can be catalyzed by the presence of trace metal and/or trace peroxide or exposure to light, heat, or base.

Propagation involves formation of a peroxy radical, which can then abstract protons to form a hydroperoxide or add to a double bond to form an addition

product radical. Hydroperoxides can decompose in the presence of free radical initiators to form peroxy and alkoxy radicals. The alkoxy radical can abstract protons to form alcohols or react with oxygen to form the corresponding acid, aldehyde, or ketone, which may react further. Termination involves two radicals combining to form stable molecules.

Inhibiting the oxidation of labile active ingredients involves preventing exposure to oxygen and light through use of a suitable barrier; sparging solution formulations with inert gas (such as nitrogen, carbon dioxide, helium) is also employed in specific cases. The inclusion of an antioxidant system such as a chelating agent to remove any free metal ions and labeling with a lower storage temperature may help. Addition of an antioxidant system, to preferentially remove oxygen or scavenge for any free radicals, may also stabilize a formulation that is prone to oxidation. If an antioxidant system is added to aid stability performance, an assay for antioxidant content is usually performed to monitor its continued effectiveness. Where the use of excipients that are prone to auto-oxidation is required, the selection of grades that contain minimal levels of aldehyde or peroxide contaminants will help to limit oxidative degradation.

Photolysis Photolysis involves degradation initiated or catalyzed by electromagnetic radiation (artificial or natural light). In a photolytic degradation mechanism, energy absorbed by the molecule is sufficient to activate degradation [12]. Oxidation, reduction, isomerization, rearrangement, decarboxylation, dealkylation, dehalogenation, and dehydrogenation pathways can all occur. Aromatic and conjugated heterocyclic molecules, aldehydes, and ketones are all susceptible. Complex mixtures of degradation products may result.

One description of photodecomposition is light-induced free radical-initiated breakdown, which is not mediated by molecular oxygen. Normal sunlight or artificial light can initiate photolytic degradation. If the electronic spectrum of a molecule overlaps with the spectrum of sunlight or artificial light, energy can be absorbed, and the molecule reaches a higher or excited state (electron promotion to a higher orbital, making the molecule more reactive). The excited state either dissipates the energy—excited electrons returning to their original orbital or reaction occurs by molecular decomposition or energy transfer with other molecules. Saturated molecules containing single bonds do not react in this way, but molecules containing double bonds can absorb light, so consequently aromatic carbons and their heterocyclic analogs, aldehydes, and ketones are the most susceptible. Substituted 1,4-dihydropyridine antihypertensive agents, for example, nifedipine, demonstrate this behavior, where different substituents can influence their photostability [13]. Many potential mechanisms exist often resulting in complex mixtures of degradation products.

Prevention of photolytic breakdown involves reducing the light transmitted to the active ingredient. The use of suitable protective barrier layers (opaque

plastic, low actinic amber-colored glass, aluminum), with reduced or no light transmittance, can provide some degree of protection for products susceptible to photodecomposition. In addition, colorants such as titanium dioxide or hydrated alumina lakes used in tablet film coatings and capsule shells may also behave as opacifiers, providing light protection for the underlying formulation. As seen previously with oxidation, scavenging molecules can minimize free radicals and hence inhibit the photolysis reaction and the use of low impurity excipients and chelating agents to remove any potentially catalytic metals all potentially prolong the shelf life of photolabile active ingredients. Protection can also be achieved by employing complexes such as cyclodextrins or use of surfactants to form a protective micelle. Excipients that preferentially absorb light may also be included in the formulation, such as titanium dioxide.

Isomerization A pharmaceutical molecule may be a geometric or stereo isomer. Isomerization is used to describe the process of conversion of a single stereoisomer into its conformational, optical, or geometric isomers [14]. The functionality and steric effects associated with the parent molecule are important. Any unwanted isomer may have different or potentially undesirable activity or be inactive. The unwanted isomer is usually treated an impurity/degradation product (see ICH Q6A).

Stereoisomers possess the same molecular formula and bonding arrangement but different orientations of the same groups in space. They can be geometric isomers or enantiomers. Isomerization usually occurs via a mechanism that involves an intermediate carbon, which is positively or negatively charged and resonance stabilized.

1.2.2.2 Reactive Species and Their Potential Origin

Formulating an active drug substance usually involves dissolving in a suitable solvent, dispersing in a suitable vehicle, or intimately mixing with excipients. Each additional ingredient may contain potential reactants, present as impurities, which might cause degradation. For example, polysaccharide excipients containing free aldehyde or ketone moieties (termed reducing sugars) can undergo an addition/elimination reaction with amine groups (Maillard reaction [15]). Further degradation mechanisms can therefore be available where the active ingredient interacts with the functional groups on the formulation excipients or with any extraneous contaminants or impurities present in the excipients (e.g., residual metals, aldehydes, or epoxides [16, 17]). In addition, more than one active pharmaceutical ingredient may be combined in the same formulation.

Excipients may not react themselves, but if they are hygroscopic, this may pull water into a formulation containing a moisture-sensitive drug. Similarly, a hydrated excipient might lose its water of hydration on mechanical processing,

Table 1.1 Origin of extraneous contaminants that may influence stability.

Potential reactive species or catalyst	Potential source
Water	Fairly ubiquitous, bound, or unbound
Acidic species	Poly(oxy)ethylenes including PEGs
Alkaline species	Dicalcium phosphate, some stearates
Metal ions	Manufacturing plant, talc, and other silicates, poly(oxy)ethylenes
Leachable materials (e.g., phthalates or volatile organic hydrocarbons)	Manufacturing plant, transfer lines, seals, containers, and closures
Antioxidants	Oils/lipids, magnesium stearate, plastic, and rubber contact materials
Peroxides	Povidones, tweens, poly(oxy)ethylenes
Aldehydes	Starch (formaldehyde), lactose, and poly(oxy)ethylenes

and this water may act as a reagent or transport medium facilitating other reactions, particularly if the drug substance is manufactured in its amorphous form or contains localized amorphous regions [18]. Table 1.1 shows some potential degradation reactants or reaction initiators and their pharmaceutical origins. Trace metals or peroxides could act as catalysts in the formation of free radicals. Extremes of pH can potentially leach potential reactants out of the surfaces associated with manufacturing equipment such as trace metals from high grade stainless steel or silicates from glass.

The potential for excipients to be a source of reactive species emphasizes the need to characterize their impurity profile extensively and to conduct compatibility studies to select suitable excipient combinations.

1.2.2.3 Environmental Factors

One of the key external factors that can affect stability is the prevailing temperature and humidity conditions associated with the intended marketing territories. On the basis of their prevailing yearly climate and mean kinetic temperature, the world can be divided, broadly, into four sectors or *climatic zones*; see Table 1.2 [19, 20]. The ICH tripartite regions are represented by climatic zone II. Representative long-term storage conditions for each zone have been derived and can be used in stability protocols to underwrite a shelf life in the specific region. A single long-term storage condition (30°C ± 2°C/65% RH) for climatic zones III and IV was originally recommended in ICH Q1F. Following further discussion some countries required specific long-term storage conditions, leading to the withdrawal of ICH Q1F. The recommended long-term storage requirements for regions represented by climatic zones III and IV are now defined in the WHO stability testing guidelines [3].

Table 1.2 Climatic zones.

Climatic zone	Description	Derived long-term storage condition
Zone I	Temperate	21°C ± 2°C/45% RH ± 5% RH
Zone II	Subtropical and mediterranean	25°C ± 2°C/60% RH ± 5% RH
Zone III	Hot and dry	30°C ± 2°C/35% RH ± 5% RH
Zone IVa	Hot and humid	30°C ± 2°C/65% RH ± 5% RH
Zone IVb		30°C ± 2°C/75% RH ± 5% RH

The local external environment such as the factory, the warehouse, and the hospital and community pharmacy shelf all will usually have some degree of associated temperature control and/or monitoring. Road or air freight transport conditions can present a concern if they result in an uncontrolled environment with respect to temperature, pressure, and vibration. Transportation conditions can be controlled to some degree but at increased cost. The concept of good distribution practice (GDP) has been introduced to cover the warehousing and wholesale distribution of pharmaceuticals including guidance on quality systems and storage/transportation practice [21, 22]. The domestic environment is usually perceived as a much less controlled environment, and the medicine should be designed to withstand the environmental conditions and physical stresses typically encountered during normal storage and use by the intended patient population.

Another aspect of the active ingredient, usually considered as part of the preclinical screening process, is the stability of potential new chemical entities under local physiological conditions during administration, for example, the low pH of the stomach for oral administration and high humidity conditions in the nasal cavity or inside the lungs.

1.2.2.4 The Environment Inside the Primary Pack

As primary packaging materials can affect the environment to which the medicine or active pharmaceutical ingredient is exposed, the influence of the primary pack on stability performance must be clearly understood [23]. The primary product packaging and the moisture content of the formulation constituents predominantly influence the prevailing equilibrium conditions inside the product pack. The product pack is important as it determines how much water vapor or any other volatile species can be transferred to/from the external environment. Any moisture ingress into the pack becomes available for sorption by the formulation. Hygroscopic formulation constituents can increase the driving force for moisture transport into the pack. As packaged medicines will tend to reach equilibrium with their external surroundings, the permeability of the packaging component materials will influence the time taken to reach this equilibrium. For example, the use of low permeability

induction seals can prolong equilibration time for solid drug products with screw-topped caps/snap on lids. Plastic blisters have some degree of associated permeability, slowing down the time to reach equilibrium further. The permeability can be expressed as a moisture vapor transmission rate (MVTR). If the MVTR for the packaging material is known, it can be combined with the humidity-adjusted Arrhenius equation (the parameters of which are determined using an iso-conversion approach in an accelerated stability assessment program or ASAP) to predict the stability of the product as a function of pack and storage condition. The presence of desiccant can also be modeled in terms of moisture take-up and saturation point [24]. See Chapter 4 relating to ICH Q1E for further examination of these factors.

Sealed aluminum foil blisters not only prevent moisture transfer but can also trap any moisture present in the environment during sealing. As the most protective packaging materials are more expensive and therefore add to the overall unit cost of the product, manufacturers need to strike an acceptable balance between the protection and cost elements of the pack. The use of desiccant can maintain a lower relative humidity by preferentially absorbing any moisture present. This equilibrium is usually reached fairly rapidly in relation to the overall shelf life. The amount of desiccant required for a specific pack size can be calculated using a simple calculation [25].

For this reason the guideline requires the assessment of stability in the actual container closure system proposed for long-term storage and distribution or as a minimum a pack that simulates its' characteristics.

1.2.2.5 The Formulation Microenvironment

Pharmaceutical products are mixtures of active ingredient and excipients. They can be considered on a particle level in terms of the formulation *microenvironment*. The local conditions in the region near to the particles of active drug substance can influence stability behavior. For example, the presence of localized moisture may dissolve any drug substance or soluble excipient present and therefore increase molecular mobility and potential for degradation reactions. Solubilization may also alter local pH and therefore reactivity [26].

In the solid state, factors such as crystal lattice defects, the presence of specific impurities or contaminants, or localized amorphous regions, may result in localized high energy "hotspots" where active ingredient molecules are more mobile and therefore more likely to react [27]. The external environment, in terms of temperature and the potential for transfer of moisture, may also affect the formulation microenvironment. Ideally a comprehensive understanding of the microenvironment factors that can affect overall stability performance should be developed.

With an enhanced level of understanding, potential changes to raw materials or process parameters can be risk assessed against their impact on the formulation microenvironment. For example, the effect the relative humidity

associated with the pack and formulation microenvironment has on solid-state degradation reaction rate is relatively well understood. This interdependency is explored further in Chapter 4.

1.2.2.6 Chemical Degradation Reaction Energetics

For a drug to degrade there needs to be a pathway or mechanism resulting in the transformation of the active to the degradant, favorable thermodynamics (a driving force for the change to occur), and favorable kinetics for the reaction to reach equilibrium in time to have a substantive and ultimately negative effect on product quality.

1.2.2.6.1 Degradation Reaction Thermodynamics

For the simplified reaction, drug → degradation product:

$$k_{\text{Rate constant}} = \frac{\text{Concentration of degradation product}}{\text{Concentration of parent drug}}$$

As the most stable state is the one of minimum energy but maximum entropy or disorder, thermodynamics predicts that a degradation reaction will not occur unless the free energy change ΔG is negative. The Gibbs free energy can be calculated as follows:

$$\Delta G = \Delta H - T\Delta S$$

where T is the temperature (K), the enthalpy change ΔH represents the difference in bond energies between reactants and products, and entropy ΔS refers to the degree of disorder.

Enthalpy effects can dominate, but entropy effects can be more significant if breakdown results in a greater number of molecular fragments or a constrained ring system opens during a degradation reaction.

The Gibbs free energy is related to the reaction rate constant via the equation

$$-\Delta G = RT \ln K_{\text{Rate constant}}$$

where R is the ideal gas constant (8.314 J/K/mol) and T is the temperature (K).

1.2.2.6.2 Catalysis

A negative value in free energy does not necessarily mean a reaction will occur. Transition state theory postulates that reactions pass through an activated complex or transition state with a higher free energy, which is then subsequently converted to the products. A catalyst can lower the activation energy, making it easier to pass through the transition state to the degradation product (Figure 1.2).

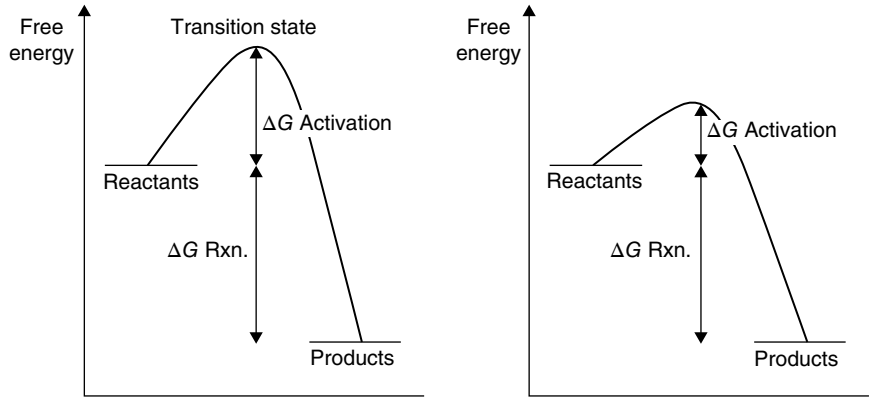


Figure 1.2 Free energy diagram for a degradation reaction.

Electron delocalization may stabilize the resonance of the transition state, facilitating its formation and increasing the likelihood of degradation. This occurs in the light-induced aromatization of 1,4-dihydropyridine-based pharmaceuticals such as nifedipine to form pyridine-related photolytic degradation products. As seen earlier, pharmaceutical excipients, manufacturing equipment, and container closure surfaces may all be sources of trace metals that could catalyze a degradation reaction. Specific drug substances, for example, mycophenolic acid, may chelate trace metal contaminants that can facilitate degradation or result in loss of potency.

Thermodynamics predicts the concentrations of degradant and reactant on reaching equilibrium (if a suitable reaction pathway exists between them) but does not predict how quickly the reaction will reach this equilibrium.

1.2.2.6.3 Degradation Reaction Kinetics

Consider the simple reaction: drug ⇄ degradation products.

If the rate of change of concentration for the drug can be measured, a rate equation can be constructed for the reaction where the rate constant k for a particular temperature and the way the concentration $[D]$ varies depends on a, the overall order of reaction:

$$-\frac{\mathrm{d}[D]}{\mathrm{dt}} = k[D]^{\mathrm{a}}$$

Reaction order can be determined experimentally by making suitable plots of concentration versus time, at a constant temperature, and from these plots the rate constant can also be calculated.

Rate models can be worked out by setting up the differential equation for change in concentration and solving the resulting integral.

For a zero-order reaction, rate is independent of concentration and a plot of concentration against time will reveal the zero-order rate constant.

Likewise for a first-order reaction, rate decreases with time as drug concentration decreases. From the concentration/time profile, we can plot the natural log of concentration against time to yield the first-order rate constant.

Substituting 0.9 for D/D0 allows us to predict the time for which 90% of the active remains (denoted as t_{90}) or 10% degradation has occurred. As this level of change would be the maximum acceptable, t_{90} can be used as an indicator of the potential shelf life that might be expected.

For a second-order reaction, the rate is proportional to the square of the drug concentration where the drug reacts with itself or with a stoichiometric amount of a reactant R. If R is in excess, for example, hydrolysis in aqueous solution, water is in great excess, and therefore the concentration does not appear to change hence the reaction effectively obeys first-order kinetics and said to be pseudo-first order.

In general, zero-order kinetics can be assumed for typical quantitative critical quality attributes such as assay and degradation product levels. This simple assumption forms the basis for statistical assessment of stability data [28].

1.2.2.7 Linking Reaction Rate to Storage Temperature

If degradation data is modeled using appropriate kinetic models and a good fit is achieved, the order of reaction can be obtained, along with a value for the rate constant. This allows prediction of the extent of degradation at a specific temperature.

Within certain constraints the Arrhenius equation can be used to calculate activation energy and predict a rate at different temperatures. The Arrhenius equation predicts the relationship between reaction rate and temperature and forms the basis for accelerated stability testing and shelf life extrapolation:

$$k = Ae^{-E_a/RT}$$

where k is the reaction rate constant, A is the Arrhenius constant, E_a is the activation energy for the specific degradation reaction, R is the general gas constant, and T is the absolute temperature (K).

The equation can be presented in a number of different forms, for example,

$$\ln k = \ln A - \frac{E_a}{RT} \text{ or } \ln \frac{k_1}{k_2} = -\frac{E_a}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

This illustrates how knowledge of a degradation rate at one temperature is used to predict a rate at a different temperature if the activation energy is known (or can be estimated) and a simple linear relationship is assumed.

The relationship between reaction rate and temperature can be visualized using the Arrhenius plot (Figure 1.3).

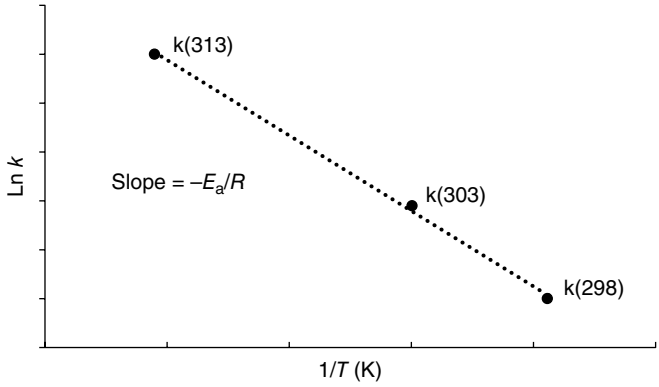


Figure 1.3 Components of the Arrhenius plot.

A linear Arrhenius plot assumes constant activation energy and the same degradation mechanism across the temperatures of interest. Changes such as solubility increase, melting, solvent losses, and formation of volatile degradation products will complicate the model and potentially result in a nonlinear Arrhenius plot. More recently a modified version of the Arrhenius equation has been developed that can be applied to pharmaceuticals to take some of these factors into account and produce much more accurate predictions (see Chapter 4).

Examples of activation energies for typical degradation processes are 85–120 kJ/mol for oxidative breakdown and 60–80 kJ/mol for hydrolytic breakdown [29].

In the absence of definitive activation energy data, assumptions can be made about activation energy, and this can be used as a means of estimating a room-temperature shelf life from higher temperature data. This is often carried out in the early phases of development, when long-term room-temperature data is not available, by extrapolating a predicted shelf life at room temperature from higher temperature data and supporting this extrapolation with subsequent real-time storage stability data.

The simplest of these methods is to apply a "rule of thumb," for example, that a 10°C change in temperature will cause a two- or threefold change in the degradation reaction rate. For example, if supporting forced degradation work indicates the active ingredient is relatively stable and no measurable changes are observed when the product is stored at 50°C for 3 months, a 12- or 18-month shelf life at 25°C could be predicted.

Another approach for general shelf life prediction purposes is to use a lower assay limit or upper limit for degradation as a shelf life target and use available reaction rate data and different estimates of activation energy to make shelf life predictions. This activity can be illustrated graphically or in tabular format.

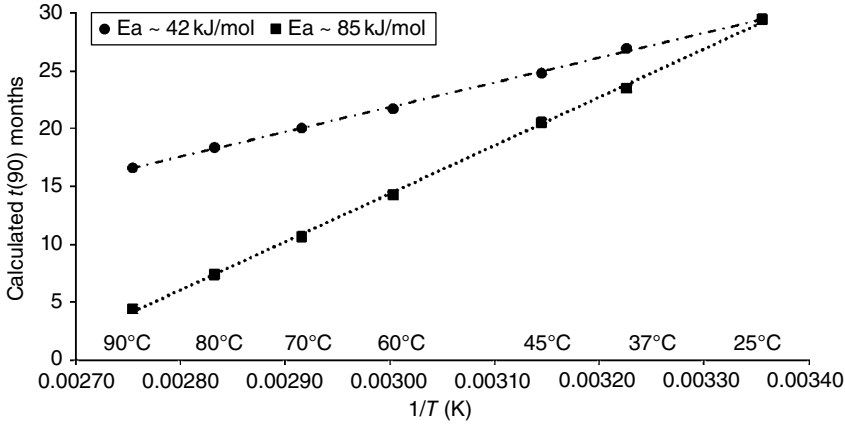


Figure 1.4 Calculation of t_{90} for a specific degradation reaction, using a high and low estimate for activation energy (E_a).

The basis for this method of extrapolation can be illustrated graphically by plotting the predicted time for the product to degrade to 90% of its initial value (t_{90}), against the reciprocal of the storage temperature in Kelvin by using an estimated value for the activation energy for the degradation reaction into the Arrhenius equation.

For example, Figure 1.4 (adapted from reference [30]) develops a shelf life prediction based on obtaining a t_{90} of 3 months for 50°C storage. Using a low value for activation energy for a particular degradation reaction, for example, 42 kJ/mol, a 3-month storage at 50°C is equivalent to about 11-month storage at 25°C. Choosing a higher value for activation energy 85 kJ/mol (a bigger activation energy "barrier" to overcome for the reaction to occur), a 3-month storage at 50°C predicts a shelf life of about 39 months at 25°C.

Another method of applying the Arrhenius equation is to calculate data in tabular format. Using the same assumptions for a lower and upper limit for activation energy, a "stress" or "kinetic equivalence" table can be constructed. For example, the potential stress temperatures and associated duration of the stress test required to predict the shelf life available if refrigerated storage is used can be calculated; see Table 1.3 [31].

These examples demonstrate the different ways the Arrhenius equation supports the prediction of long-term shelf life from short-term data generated at higher temperatures.

The concept of "stress equivalence" derived from the Arrhenius equation underpins the stability protocols and storage conditions described in the stability guideline where higher (accelerated) temperatures are emphasized early in the protocol, while lower temperature storage is extended for the full duration of the target shelf life (Table 1.4).

Table 1.3 Predicted shelf life for refrigerated storage.

Duration of stress test (days) required to predict stability under refrigerated storage (5°C) for the specified duration								
	6 months		1 year		2 years		3 years	
Temperature (°C)	85 kJ/mol	42 kJ/mol	85 kJ/mol	42 kJ/mol	85 kJ/mol	42 kJ/mol	85 kJ/mol	42 kJ/mol
14.5	55.3	100	111	201	221	402	332	603
25	15.1	54	32	108	64	217	97	326
35.5	5.1	30.6	10	61	20	122	31	183
47.5	1.5	16.6	3	32	6	66	9	100
60	0.5	9.2	0.9	18	1.9	37	2.8	55

Table 1.4 Typical stability protocol.

Storage condition	Storage time (months)						
	1 month	3 months	6 months	9 months	12 months	18 months	24 months
40°C/75% RH	X	X	X				
30°C/65% RH	X	X	X	X	X	X	
25°C/60% RH	X	X	X	X	X	X	X

1.2.2.8 Physical Stability

While a detailed discussion on physical stability does not form a substantial part of the guidance, potential changes to the physical attributes of the input drug substances and excipient can also influence the stability of a medicine. For example, as solubility is dependent on the physical form of the drug, critical performance attributes, including bioavailability and therefore the quality safety and efficacy, can be affected.

Similarly physical changes to an excipient present in the formulation may affect the performance of the medicine. It is important to understand the different physical states available to a drug and the associated excipients in the final dosage form. The thermodynamically most stable state is arguably the most critical as other physical states will tend to transform to this state. Similar to chemical degradation reactions, molecular mobility, and any factors that increase molecular mobility in the solid state are critical as to whether a physical transformation of the parent molecule occurs. The Gibbs free energy difference between the different physical states dictates whether the transformation is likely to occur.

Classic examples of shelf life limiting physical changes include crystallization of amorphous drug substance, changes to crystal habit on storage [32], and disproportionation of a salt [33]. If a drug substance can exist as different crystalline forms (polymorphs), each one will have a different free energy depending on prevailing local temperature and humidity conditions, and therefore transformations between polymorphs may profoundly affect solubility (and therefore bioavailability). If a drug exhibits polymorphism, its physical state should be monitored during stability testing (see ICH Q6A [34]). As the presence of water can facilitate molecular movement, it can cause physical form transformation; therefore moisture absorption and moisture content should also be monitored so that any change to hydration status is detected. The kinetics of solid phase transitions is highly complex, making prediction and extrapolation more difficult to achieve.

Selection of the most appropriate physical form involves screening work to understand the number of different physical forms available to the parent molecule and takes place early in the development lifecycle. This is often performed using screening tools after short-term stress storage to accelerate any potential transformation. The screening tools include solid state spectroscopy such as Fourier transform infrared spectroscopy (FTIR), near-infrared (NIR), Raman spectroscopy, X-ray powder diffraction (XRPD), solid-state nuclear magnetic resonance spectroscopy (SS-NMR), and thermal methods including differential scanning calorimetry (DSC) and Brunauer–Emmett–Teller (BET) surface analysis to measure specific surface area and microcalorimetry. Such screening experiments may assess the following aspects:

- Are hydrates or solvates formed in contact with water, solvent, or mixtures of both?
- Are different polymorphic forms available when the parent molecule is recrystallized from a variety of different solvents?
- What physical conditions in terms of temperature and humidity cause a transition between the available forms?
- Does the salt disproportionate?
- Do particle size reduction processes such as milling or micronization have an effect?

The data generated should allow the rational selection of the most appropriate physical form for further development, ideally the most thermodynamically stable option. Where it is demonstrated that different physical forms are available, stability assessment should normally include physical form testing.

In practice automated screening approaches, involving preparation and evaluation of multiple combinations and conditions simultaneously, can be particularly effective. The performance of screening experiments in such a systematic way is an efficient process to check if a potential drug molecule can exist in more than one physical form and to get a preliminary comparative assessment of the stability of drug substance salts or to assess prototype formulations. The sequential nature of degradation studies and excipient compatibility work lends itself to the use of array systems with temperature and humidity control within individual study vials. Using transparent containers the contents can be probed nondestructively using IR or Raman spectroscopy and visible assessment prior to further chromatographic physical forms. These workflow systems can also be used to assess the potential for a molecule to exist in different physical states and examine the physicochemical performance of alternate drug substance salt forms and drug excipient mixtures or formulation prototypes in automated experiments (see Figure 1.5).

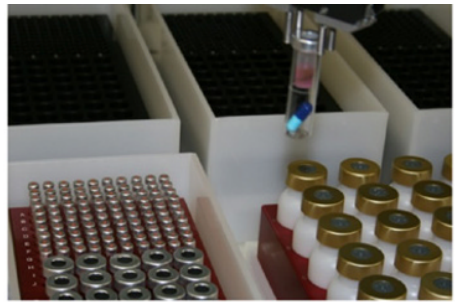




Figure 1.5 Automated system for rapid stability screening.

1.2.3 The Fundamental Science That Underpins Stability Testing: Summary

To summarize, the key elements relating to the science underpinning stability include

- Molecular structure and functionality of a compound can strongly influence its reactivity.
- Functional groups influence the electron distribution around a molecule resulting in regions susceptible to attack.
- Functional groups can also stabilize intermediates or activated complexes that increase the rate of reaction.

- Formulation excipient and manufacturing processes can act as sources of potential degradation reactants or reaction initiators, for example, metal ions.
- Local (internal) and external environments can significantly affect chemical degradation
- Thermodynamics and kinetics dictate whether and how fast degradation occurs.

1.3 Part II: Practical Application of the ICH Stability Guidance

1.3.1 General Considerations

The guidance covers some of the practical aspects of stability testing, for example, consideration of the technical capability of storage facilities used for stability samples in terms of temperature and humidity control.

Local operating procedures should cover the practical stability study aspects such as

- Responsibilities and accountabilities for carrying out the stability study
- Sampling of batches for stability testing
- Pull time and analysis windows, acceptable maximum duration between manufacture date, and stability study start date
- Amount of material placed on stability including the overage to account for sample retest or other additional analysis
- The data integrity processes that ensure the reliability, accuracy, and traceability of all stability data generated in support of clinical or commercial license applications

These study details are compiled within a stability protocol along with clear accountabilities for management and execution of the study. The protocol is usually formally reviewed and approved and forms part of the overarching quality management system that should cover the general operation and compliance aspects of study execution in order to meet the requirements of good manufacturing practice principles; see, for example, ICHQ10 [6]. A key part of the quality system is a defined procedure for dealing with unexpected study outcomes, such as "out of trend (OOT)" and "out of specification (OOS)" results including the process for confirming an unexpected stability test result, which may involve resampling and retest. The importance of stability data, particularly in late phase development, and the commercial lifecycle means that a continuity/recovery plan should be in place to mitigate any failure of stability storage facilities or associated infrastructure. Secure data storage and backup is also important.

Stability tests should always be performed on material that is fully representative of the medicines that patients will use both in a clinical and commercial setting. Attributes that are relevant to quality, safety, and efficacy are usually tested as part of the stability protocol. During stability testing, the

material under investigation should remain within its target specification and not exhibit significant change. Additional ICH guidance provides useful input to critical attributes that should be included in the control specifications, which assure quality, safety, and efficacy, and should therefore be considered when assembling a stability protocol. For example, ICHQ6A [34] details typical tests and specification limits for drug substance and drug product, while ICH Q3A to Q3D focuses on impurities [35–38]. This guidance is discussed further in Chapters 13 and 16.

While the objective of the stability study is to ensure that the material under investigation will remain within specification during its assigned retest or expiry period, the study should also seek to identify any underlying trends or any changes that could be considered to be significant.

As stability testing underpins the whole lifecycle for a pharmaceutical product, the analytical methods used must be suitable for use throughout this lifecycle, with appropriate controls and monitoring to assure continued fitness for purpose (see ICH Q2). The term stability-indicating implies that a method has the required specificity to precisely and accurately quantify the active species and any degradation products. The method should be capable of reliably quantifying change compared with specification limits or stability study targets. For quantitative methods, the ability to distinguish a real change on stability from the normal analytical variability associated with the method is important [39]. To ensure the method is capable, suitable precision and bias studies, underpinned by validation characteristics such as linearity, specificity, and limits of quantification and detection should be performed. An understanding of the capability of the method in terms of precision will inform the number of test replicates required to assess a specific stability attribute. Considering that the method will need to maintain its fitness for purpose potentially for several decades, an understanding of the impact of adjusting input reagents and instrument parameters early in the method lifecycle will pay dividends when change occurs later on [40]. Where the parent compound and degradation products contain a UV chromophore, chromatographic methods with UV detection are used extensively for stability testing in particular high performance liquid chromatography with UV detection (HPLC-UV) or ultra-high performance liquid chromatography (UHPLC-UV). Where the parent molecule and main degradation products do not contain a UV chromophore, other methods such as gas chromatography with flame ionization detection (GC-FID) or hyphenated techniques allying chromatographic separation with detection using mass spectroscopy (HPLC-MS or UHPLC-MS) should be considered.

The ICH guidance is intended to support the generation of appropriate labeled storage conditions and shelf life for commercial license applications. However, elements of the guidance can be taken into account when assembling stability protocols in the earlier phases of development to ensure that nothing unexpected emerges during the formal studies performed subsequently. As not all development candidates progress beyond the early safety assessment and

proof of clinical concept stages, there is a balance to strike between efficient stability approaches and more extensive studies that will yield supporting information that can inform subsequent formal protocols.

Points to consider when assembling a stability testing protocol include the target clinical and/or commercial shelf life, which informs the overall duration of the study. Other business-related aspects may also be considered, for example,

- Inventory control and the associated warehousing needs.
- Production cycling, the number of batches are likely to be manufactured per year.
- Distribution logistics for the new medicine.

The selected stability packaging should be representative of the presentation intended for the commercial market.

ICH requirements form the core of any stability protocols intended for global registration. Additionally specific regional guidance may detail further requirements such as alternative storage conditions (e.g., 30°C/75% RH), specific data formatting requirements, the number of batches required, the extent of bracketing and matrixing permitted, and the need for site-specific or country-specific stability testing [41]. It is therefore important to understand where regional requirements differ from the ICH guidance, engage with the relevant regulatory body, and, when needed, develop the global protocol further to encompass any additional requirements.

1.3.2 Drug Substance Stability

1.3.2.1 General Considerations

A fundamental understanding of the intrinsic stability of the input drug substance(s) for a medicine is the first step in developing the stability knowledge that translates to the demonstration of satisfactory behavior under normal and accelerated storage conditions required for the registration of a medicine.

A retest period is usually assigned to drug substance, within which it is expected to comply with specification. If a drug substance batch is required for drug product manufacture beyond its labeled retest date, it should be retested to confirm continued compliance with specification prior to use.

During development the synthetic route and manufacturing process for a drug substance will be optimized and scaled up. Changes to the synthetic route and/or process are usually risk assessed to evaluate any impact on parameters that are critical to drug substance stability. Chemical, physical, biological, and microbiological impacts usually form part of this assessment. The outcome of the assessment should inform a decision whether to test an additional batch that is representative of the changed process.

While not covered in the stability guidance, similar principles may be applied to structurally contributing raw materials and intermediates used in the production of drug substance. The data generated can underwrite short-term storage and transportation of these materials if this is required for operational reasons.

1.3.2.2 Early Stages of Development

ICH Q1AR(2) indicates that knowledge of the intrinsic stability of a new molecule and the associated potential degradation pathways, combined with an understanding of the degradation products that are formed under accelerated and long-term storage, forms an important part of the licensing application. Accumulation of this knowledge usually starts in the early stages of development with a theoretical consideration of the behavior of the individual functional groups that constitute the molecule and the likely local physical and chemical conditions that they may encounter. The exercise can aid in the selection of the experimental conditions to use in the subsequent practical experimentation to understand which degradation pathways are relevant. The theoretical exercise can also aid in the deselection of any formulation excipients or packaging components that would be incompatible. Knowledge of organic functional group behavior, predictive algorithms, and degradation product databases are all useful inputs. The predictive exercise informs the selection of suitable conditions to use when developing forced degradation study protocols.

The theoretical assessment is then followed with practical experimentation, usually involving conditions selected to rapidly initiate a specific degradation pathway and are in excess of the accelerated storage conditions described in the guidance. A high level overview of this activity is provided in ICH Q1AR(2). These studies are designed to assess influence of acid/base, thermal stress, oxidative challenge, and photolytic challenge. The influence of environmental conditions (e.g., pH, temperature, humidity, and light) is also assessed. The guidance suggests a single batch of drug substance is subjected to thermal stress at 10°C increments above the accelerated storage condition, humidity stress above 75% relative humidity, oxidative, and photolytic challenge. As hydrolysis is one of the most common degradation mechanisms, the guidance also indicates that this mechanism should be explored by assessing the drug substance in aqueous solution or suspension in addition to the high humidity solid state challenge. Organic cosolvent can be used to dissolve poorly soluble drug substance, but this should be carefully considered as these may influence the outcome of the experiment. The actual conditions used will depend on the intrinsic stability of the drug substance but should be selected carefully so that they produce meaningful levels of degradation, that is, to ensure the pathways highlighted are the ones most likely to be encountered under real-time and accelerated storage [42]. Additionally, the studies should be designed to promote degradation at a fast enough rate to produce quantifiable levels of the degradation products most likely to be formed under real-time and accelerated storage. A few iterations may be required to form the required level of overall breakdown. The use of excessive amounts of stress risks emphasizes nonrelevant degradation pathways [43]. Details on the recommended photostability testing protocol and storage conditions are provided in ICH Q1B (see Chapter 2 ICH Q1B).

The resulting degraded material is then examined to identify the most prevalent degradation product using appropriate structural elucidation tools. The use of suitable identification thresholds is recommended to avoid expending extensive effort on structural elucidation of degradants that are unlikely to form under normal storage and transportation conditions. Before starting the degradation study, suitable identification thresholds should be derived. Three potential strategies to establishing identifications thresholds are as follows:

- Confirm the identities of only those degradation products observed from long-term and accelerated storage.
- Apply an identification threshold based on percentage of total degradation and/or largest response observed in the degraded analyte.
- Use a scaled approach based on established identification threshold practices, for example, ICH Q3B identification thresholds [44].

When examining the degraded solutions, an assessment of "mass balance" is instructive. This involves comparing the total level of degradation products detected with the remaining level of parent substance with due consideration of the precision of the analytical quantification methods used. Ideally the summation result should approach 100%, but this is dependent on the nature of the degradation products formed (i.e., presence of absence of a UV chromophore in the degradant) and the capability of the quantification methods. To fully characterize the degraded material, different detection platforms may be used in tandem such as HPLC-UV and HPLC-MS and GC-FID or GC-MS to maximize the number of degradation products detected and quantified. The degraded material or solutions can be used as a test analyte to develop suitable routine analytical methods and confirm their specificity and therefore "stability-indicating" capability. The preliminary studies help to define the content of the subsequent stability protocols, in particular selection of the most likely long-term stability storage condition (room temperature, refrigeration, or freezer storage).

The objective of the development stability program is to support the clinical and safety program and generate information that will inform subsequent formal protocols. During the earlier stage of development, stability testing is undertaken to ensure that the drug substance complies with the specification used to underwrite use in phase 1 or phase 2a clinical studies and establish a preliminary retest period and storage recommendation to support these studies. The studies may also help to confirm the suitability of the selected primary and secondary packaging (e.g., polyethylene bags stored inside high density polyethylene drums).

During early development the application of long-term, intermediate, and accelerated storage conditions are used along with additional conditions to act as stress challenge to generate some of the fundamental supporting information referred to in later regulatory filings. These data help to develop a wider

Table 1.5 Example early-phase protocol for drug substance.

Storage condition	Storage time (months)					
	1.5 months	3 months	6 months	9 months	12 months	24 months
25°C/65% RH	—	X	X	(X)	X	X
30°C/65% RH	—	(X)	(X)	(X)	(X)	(X)
40°C/75% RH	—	X	X			
50°C	X	X	(X)			

(X), optional.

knowledge of the drug substance and its associated process. A single batch, representative of the material being used in the clinical program, in a representative container, will usually be set down on a slightly more expansive protocol (e.g., inclusion of additional stress test conditions) compared with the ones described in ICH Q1A(R2). Table 1.5 shows an example of an early phase protocol.

The testing performed should assess the critical attributes that underpin the quality, safety, and/or efficacy of the clinical drug substance, usually involving chemical, physical, biological, and microbiological assessment as needed. Typical test parameters for drug substance stability assessment include appearance, assay, related substances (universal tests, ICH Q6A), physical form (if different forms have been shown to exist in screening studies), water content or loss on drying, particle size distribution (if relevant to the drug product), and microbiology/water activity (specific tests, ICH Q6A).

The basis for retest date assignment for drug substance is usually based on the combination of accelerated and real-time storage. The retest date may be extrapolated and subsequently confirmed with real-time data.

The relevant knowledge outcome of the stress testing is summarized in the regulatory filing and, if appropriate, overview diagrams developed to summarize the behavior of the drug substance over the relevant degradation conditions (see Figure 1.6).

As the synthetic route and drug substance process is scaled up and optimized for commercialization, a risk-based assessment of the impact of any resulting the change should be undertaken. The main outcome of this assessment is a decision on whether a new stability protocol, testing a representative batch made using the updated process, is required. Comparison of the impurity profile for the optimized process batch with batches used in qualifying safety studies will also form part of this assessment, in particular, to assess whether additional safety qualification is necessary.

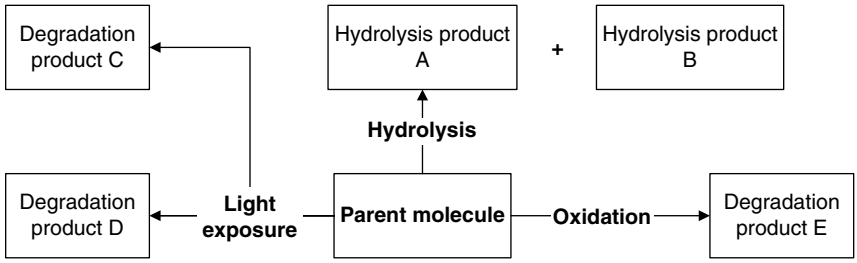


Figure 1.6 Example degradation "map."

1.3.2.3 Later Stages of Development

During the later stages of development, the formal stability studies are used to establish the retest date for phase 3 and commercial drug substance. The guidance states that drug substance stability should be assessed at different temperatures and humidities, and it recommends the most appropriate storage conditions to use. For example, for drug substance intended for storage under ambient conditions in the ICH regions, the long-term storage options are 25°C/60% ± 5% RH or 30°C/65 ± 5% RH, 30°C/65 ± 5% RH for intermediate storage, and 40°C/75% ± 5% RH for accelerated storage.

In the later stage of development, the drug substance process is optimized for commercialization. The guideline requires that three batches are evaluated as part of the stability testing that will support the registration program. These batches should be a minimum of pilot scale using the same synthetic route, a manufacturing process that is fully representative of the intended commercial-scale process and to a quality standard that is fully representative both of batches that have been use in clinical studies and the intended commercial quality. In particular the impurity profile produced from the optimized commercial process should be representative of the profile qualified in long-term safety studies. The stability study should be performed in the primary and secondary packaging intended for long-term commercial storage and transportation. Scaled-down versions of the containers may be appropriate, but the materials of construction should be the same.

If particle size control is required using further particle engineering steps, for example, micronization, usually the micronized material is set down on formal stability. To underwrite a suitable storage period or "holding time" for the un-micronized intermediate, at least one representative batch of un-micronized material is usually assessed [45]. Annealing of any process-induced amorphous regions can occur during this holding period, and this can therefore be monitored during stability assessment of the un-micronized drug substance.

The guideline recommends the storage conditions (challenging thermal stability and moisture sensitivity) and testing frequency that should be evaluated for the real-time, intermediate, and accelerated storage conditions for drug substance intended for freezer, refrigerated, and general (ambient/controlled

Table 1.6 Typical protocol for drug substance intended for long-term storage at ambient/controlled room temperature.

Storage condition	Storage conditions	Minimum recommended data available at filing	Typical protocol (optional test points at later time points may be appropriate)
Long term	25°C/60% RH or 30°C/65% RH	12 months	Initial, 3, 6, 9, 12, 18, 24, 36 months
Intermediate	30°C/65% RH and/or 30°C/75% RH[a]	6 months	Initial, 3, 6, 9, 12 months
Accelerated	40°C/75% RH	6 months	Initial, 3, 6 months

[a] Not required for ICH regions but used as long-term storage condition for climatic zone IVb territories.

room temperature) storage. The guidance indicates that a minimum of 12 months' real-time data and 6 months' accelerated data are usually required at the time of filing. The study is usually continued for the duration of the target retest period for the drug substance. A typical protocol is detailed in the following text for drug substance intended for long-term storage at ambient/controlled room temperature is shown in Table 1.6.

For drug substance, ICHQ6A (see Chapter 16) provides insight into the critical drug substance attributes therefore the tests that should be considered for inclusion in the stability protocol. Assessment of appearance and organic-related substances is routinely carried out. If the drug substance is hydrated or a solvate, levels of water and/or solvent are also routinely assessed. If different physical forms are available to the drug substance, or if the substance is a chiral molecule, these characteristics are periodically assessed to monitor for any changes on storage. Depending on the intended use, assessment of the particle size distribution of the drug substance may be appropriate. Periodic assessment of microbiological characteristics (or water activity as a surrogate, where justified) may also be performed as part of the stability assessment.

The resulting stability data is used to support a retest period proposal that will state the duration over which the drug substance will comply with the proposed specification and therefore is suitable for the manufacture of drug product. After this period, the drug substance can be retested, and if it complies, the drug substance can be immediately used to manufacture drug product.

1.3.2.4 Lifecycle

If the stability data available at filing does not include long-term storage for the duration of the proposed retest period, there should be an ongoing commitment to complete the study in the post-approval lifecycle. Stability data on a total of three production scale batches is expected, and where the initial filing

does not contain this data, a post-approval commitment to generate and provide this data is required. At least one batch of drug substance per year is set down on stability and monitored to confirm stability performance.

During the commercial lifecycle, the impact of any changes on stability performance should be formally assessed and stability studies initiated to mitigate any highlighted risk if appropriate (see ICH Q10). The changes may include input raw material characteristics or supply and alternate manufacturing sites or changes to the process for raw materials/intermediates.

1.3.3 Drug Product

1.3.3.1 General Considerations

Shelf life is defined in ICH Q1A as "The time period during which a drug product is expected to remain within the approved shelf-life specification, provided that it is stored under the conditions defined on the container label." The data generated confirms that a medicine maintains its fitness for use in terms of quality, safety, and efficacy over the duration of the labeled shelf life and during normal use by the patient in accordance with the label.

The stability protocol for the drug product will contain specific details on the representative batches selected, description of the primary and secondary packaging used, sample numbers (e.g., number of units per container), test intervals, storage conditions, target criteria for the study, and references for the specific test methods to be used. The protocol should serve not only to generate data that demonstrates drug product suitability over the proposed shelf life but also to make provision to cover potential temperature excursions during transportation and storage.

For a drug product, knowledge of the input drug substance, how the drug product will be dosed by the patient, and any specific guidance associated with product platforms (e.g., parenteral or inhalation products) all inform the product attributes to be assessed during stability testing. Specific product guidance also provides insight into what constitutes a significant change that requires further investigation or assessment of additional samples or storage conditions.

Where a container closure system uses elastomeric components, for example, stoppers for parenteral liquids and freeze dried products, or sealing gaskets in metering valves used in inhalers, the potential for interaction with the formulation should be considered as part of stability evaluation. Understanding the potential extractables from the elastomers in contact with the formulation and confirming whether any of these species appear as leachables during real-time and accelerated stability testing is recommended [46]. Evaluation of different stability storage orientations, particularly if a specific orientation maximizes elastomer and formulation contact, should be considered.

One aspect of testing that is not covered specifically in the stability guidance is the assessment of the continued suitability of a multiple-dose medicine during its in-use period after it has been removed from any protective pack or the continued suitability of multiple dose liquid or semisolid products once the primary pack is breached, particularly from a microbiological perspective [47]. For relevant dosage forms, this testing is usually performed during formal stability testing. The in-use shelf life is usually stated on the product label.

1.3.3.2 Early Stages of Development

The early stages of development present the opportunity to develop a stability knowledge base for the product or series of products. The guidance states that stability data generated on clinical formulations can be used along with the output from the drug substance stress testing and stability program to develop the protocols, (storage conditions, time points) used for later phase pivotal stability work.

In the early stages of development, studies to underwrite safety and prove clinical concept for a new medicine are performed. Generally, these studies use simpler formulations (e.g., drug in bottle, drug in capsule approaches, parenteral solutions, or delivery platforms such as nebulized solutions or suspensions). Stability testing is required to support the use of these early formulations in the clinic, and the length of the studies is dependent on the duration the safety or clinical studies and the complexity of the formulations used [48]. Microbiological assessment is usually performed for the relevant formulations, such as liquids. Representative product is set down, and the protocol will usually consider the location of the clinical studies with respect to climatic zone and any transportation and in-use factors. A preliminary assessment of photostability is prudent and, where required, any observations are mitigated by appropriate light protection (see Chapter 2). The stability data generated on the input drug substance is supportive for the simpler, drug substance-based products. Investigational protocols to cover short-term clinical evaluation can be performed on single batches of formulation, and these studies can start to develop a stability knowledge base for the product. Tracking degradation products through these studies will pay dividends later in development.

During the early stage of development input to formulation, development will commence and excipient compatibility experiments may contain a stability testing perspective. The objective is to identify any additional degradation pathways that are initiated as a result of contact between an excipient, or excipient admixtures, and the parent drug substance. This work should highlight or confirm any chemical or physical incompatibility, indicate any additional pathways available to the drug substance, and therefore aid the selection of appropriate formulation constituents. Knowledge of the intrinsic stability of the drug substance will aid the selection of the most appropriate formulation option and selection of compatible excipients. Similar knowledge of the

chemistry, functionality, and impurity profile of excipients is important and will help to highlight potential interactions between excipient and drug substance [49, 50].

Short-duration exposure to high temperature stress and accelerated storage, usually with a room-temperature reference, may be used to select the optimum excipient or look for any specific incompatibilities between drug substances and typical product excipients. A statistically designed experiment incorporating a matrix of excipients and levels will provide the most comprehensive assessment. While the initial focus is on chemical incompatibility, as the studies progress and formulations refined, physical testing may also be performed (e.g., dissolution and disintegration assessment for prototype solid dosage forms). Ideally physical form transformation of the formulated drug substance should be avoided and specific methods to assess this may be required. However where amorphous drug substances or salt forms prone to dissociation are developed, or where hydrate/anhydrate interconversion is possible, monitoring for physical form changes on stability will be required [51]. This is particularly important where specific manufacturing processes, such as wet granulation or aqueous film coating, introduce extraneous water as a reactant or mediator of physical change. Similar chemical degradation pathways to the stress testing of drug substance might be observed. The output from these studies is used in the development of the lead formulation for use in phase 2b and phase 3 clinical studies.

If the product achieves proof of concept and progresses to later development, usually using a simple enabling formulation, changes will be required to the manufacturing process and product formula in order to develop a commercially viable product. A structured risk assessment to inform the decision whether or not to set new batches on stability should be carried out.

To generate a shelf life to support investigational medicinal product, the location of the clinical study centers in terms of their climatic zone and the typical duration of the clinical studies should be considered when planning the long-term storage condition and knowing how long to assess it, respectively. Accelerated storage can also be used to cover any temperature excursions during storage and transportation.

In addition to the investigational medicinal project containing the active drug substance, a suitable shelf life will also be required for any placebo and clinical comparator products used during the clinical program. The testing protocol requirement for the placebo, or for the comparator product if the clinical study design requires it to be "blinded," would include appearance as a minimum and potentially other performance-related tests depending on the type of product.

In terms of what stability attributes to test for specific drug products, ICHQ6A (see Chapter 16) provides insight into critical performance attributes to consider including in the stability protocol. Typical stability test attributes for specific dosage forms are shown in Tables 1.7 and 1.8.

Table 1.7 Typical quality attributes critical to stability performance for oral and parenteral products.

	Pharmaceutical product		
Quality attribute	**Tablet/capsule**	**Preserved oral solution/suspension**	**Parenteral**
Visual assessment	X	X	X
Physical form	X_R	X_R	NA
Assay	X	X	X
Impurities	X	X	X
Hardness/friability	X	NA	NA
Unit weight/weight of content	X	X_R	X_R
Water content	X	NA	NA
Preservative assay	X_R	X	X_R
Dissolution/disintegration	X	NA	NA
Leachables	NA	X_R	X_R
Microbiology	X	X_R	X
Sterility/endotoxins	NA	NA	X
pH	NA	X	X
Osmolarity	NA	NA	X
Particulates	NA	NA	X
Other tests	NA	Solution $clarity_R$ or Suspension $resuspendability_R$	NA

NA, not applicable; X_R, perform test if relevant.

1.3.3.2.1 Data Evaluation

In the absence of extensive long-term stability data in the early stages of development, an extrapolated shelf life is usually assigned on the basis of the accelerated and long-term data available at that point. The extent of extrapolation depends on a series of factors:

- The complexity of the product
- The extent of prior knowledge gleaned from stress storage experiments focused on the evaluation of the intrinsic stability of the drug substance
- Relevant excipient compatibility work
- The observed accelerated and real-time stability behavior and the amount of available supporting stability data

Table 1.8 Typical quality attributes critical to stability performance for different types of inhalation products.

	Pharmaceutical product type		
Quality attribute	**Nasal inhaler**	**Dry powder inhaler**	**Metered dose inhaler**
Visual assessment of product and formulation	X	X	X
Physical form	X_R	X_R	X_R
Assay	X	X	X
Impurities	X	X	X
Dose content uniformity	X	X	X
Unit weight	X	X	NA
Water content	X	X	X
Aerodynamic particle size or droplet size distribution	X	X	X
Leachables	X	X	NA
Microbiology	X	X	X
Other tests	pH	Plume geometry	None
	Preservative $assay_R$	Actuation weight	
	Plume geometry		
Pack tests	Leakage rate	Leakage rate	NA

NA, not applicable; X_R, perform test if relevant.

Where the accelerated stability is within the target limits with no obvious trends or other concerns, extrapolation factors of three or four times the available data, to a maximum of 12–24 months, are often used. These extrapolations must be confirmed by subsequent long-term data.

The accelerated and stressed storage data generated during the early stages of development can be used to cover short-term temperature excursions during clinical use. In-use stability data can be generated to underwrite specific handling during the clinical study, where required. Temperature cycling studies can also be used to cover short-term temperature excursions during storage and transportation. For suspension-based drug products, temperature cycling studies can also be used to investigate the potential for crystal growth via Ostwald ripening and the consequent impact on drug substance particle size distribution for the product.

1.3.3.3 Late Stages of Development

During the later stage of development, the long-term safety and efficacy of the medicine will be assessed, and testing will be performed to confirm the stability of materials used in longer-term clinical studies such that the quality, safety, and efficacy is assured from the time the clinical product is released to the end of the assigned shelf life.

In late stage development, the formal studies required for licensing applications are initiated. The long-term storage conditions selected will be dependent on the climatic zone associated with the region intended for marketing.

A minimum shelf life of 24 months is the usual target for a marketed product, and the guidance recommends that a minimum of 12 months' long-term data and 6 months' accelerated data are available at filing. However for many pharmaceutical products, the target shelf life can often reliably be extrapolated on the basis of 6 months' long-term and accelerated data sets [52].

The guidance provides the recommended storage conditions for licensing applications in the ICH regions. Where registration beyond the ICH regions is required, the ASEAN/WHO intermediate storage conditions (30°C ± 2°C/75% RH) could be substituted for the ICH intermediate storage conditions (30°C ± 2°C/65% RH) deliver a more efficient "global" protocol, providing earlier development data supports the used of the selected packaging under these more aggressive storage conditions.

Three batches that are fully representative of each commercial presentation should be assessed. While at least two of these batches should be a minimum of pilot scale, the third can be smaller if justified using a manufacturing process that is also representative of the process intended for the commercial product. The smaller batch should typically be at least 100,000 units or one-tenth of the commercial manufacturing scale, whichever is larger. If stability data on the first three commercial-scale batches intended for marketing are not available at filing, the guidance recommends that these batches are placed on stability and tested under the same long-term storage protocol. The use of different batches of input raw materials, drug substance(s), and packaging components is recommended so that the product batches used in the study are indicative of routine production. Attributes that are considered critical to stability are monitored on batches under the storage conditions and time points recommended in the guidance. A typical "global" protocol is shown in Table 1.9, incorporating long-term storage conditions that cover most of the major markets.

The guidance contains a short section on stability data evaluation; when a data set is available exhibiting minimal variability and limited degradation, formal statistical analysis is not usually needed. In cases where quantitative attributes show some change with time, an approach to shelf life assignment is described where quantitative stability data sets are assessed statistically and the shelf life estimated from point at which the 95% confidence interval intercepts the proposed specification limit; see Figure 1.7.

Table 1.9 Example "global" protocol.

	Storage time (months)							
Storage condition	**1 month**	**3 months**	**6 months**	**9 months**	**12 months**	**18 months**	**24 months**	**36 months**
25°C/60% RH		X	X	(X)	X	(X)	X	X
30°C/65% RH		(X)	(X)	(X)	(X)	(X)	(X)	(X)
30°C/75% RH			X	(X)	X	(X)	X	X
40°C/75 open	X							
40°C/75% RH	X	(X)	(X)					
50°C/ambient	X	(X)	(X)					
Light challenge	X							
Refrigerated (reference)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)

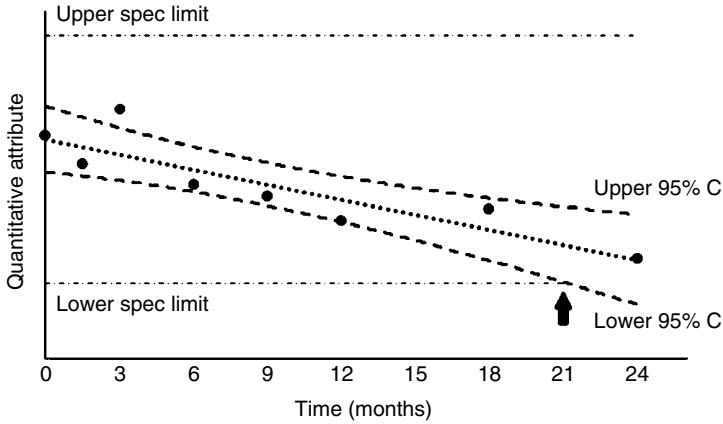


Figure 1.7 Statistical calculation of shelf life.

Further details on the recommended statistical modeling approaches are detailed in ICH Q1(E), which describes how to calculate a shelf life or retest period with an appropriate level of confidence [53] (for more information, see Chapter 4). A "poolability" test, to combine the data sets from the three representative batches, is also described. Normally real-time data supported by accelerated data is used to calculate the shelf life. These calculations should establish the storage time for which the attribute critical to stability will be acceptable for all future batches manufactured, packaged, and stored under similar conditions.

1.3.3.3.1 Bracketing and Matrixing

Where a range of products, pack sizes, and units per container are to be assessed, bracketing and matrixing approaches may be justified [54]. For more information, this topic is discussed in Chapter 3. These approaches are not used extensively in the early stages when trying to build the knowledge base but can save considerable time and effort later in development.

Where a range of product strengths have been developed based on a similar composition (e.g., a range of tablet strengths manufactured by different compression weights of a common blend), bracketing describes the practice of performing full stability testing on the extremes of the product range. Bracketing can also applied where different numbers of units are filled into the same primary container. This results in a reduced study design.

Matrixing is another stability study reduction strategy and is based on the assumption that testing a subset of all samples at a particular time point is fully representative of all samples at that time point. A subset of the total samples is tested at a specified time point, and a different subset tested at a subsequent time point, with all samples tested at critical time points such as initial and target shelf life.

Bracketing and matrixing designs should be scientifically and technically justified, and depending on the complexity and degree of reduction, it is prudent to consult with regulatory stakeholders before the studies are initiated.

1.3.3.4 Lifecycle

When the submitted file does not contain long-term stability data on three full-scale production batches for each presentation, a post-approval commitment is required during the commercial lifecycle to provide these stability data sets. The protocol applied should replicate the one in the original filing. The submitted file should also include a commitment to verify stability performance periodically during the commercial lifecycle (also termed annual stability commitment testing).

During the commercial lifecycle, the impact of any changes on product stability performance should be formally assessed and stability studies initiated to mitigate any highlighted risk if appropriate. The changes may include excipient characteristics or supply, changes to primary packaging materials or supply, alternate manufacturing sites, or improvements to the product manufacturing the process.

During the commercial lifecycle, registration of the medicine in other territories outside of the ICH regions may also be planned. Data will be required at the long-term stability condition derived from the mean kinetic temperature representative for the region. The required data may already be available from existing or ongoing studies. It is recommended that any specific regional stability requirements are compared with existing data sets, and where significant

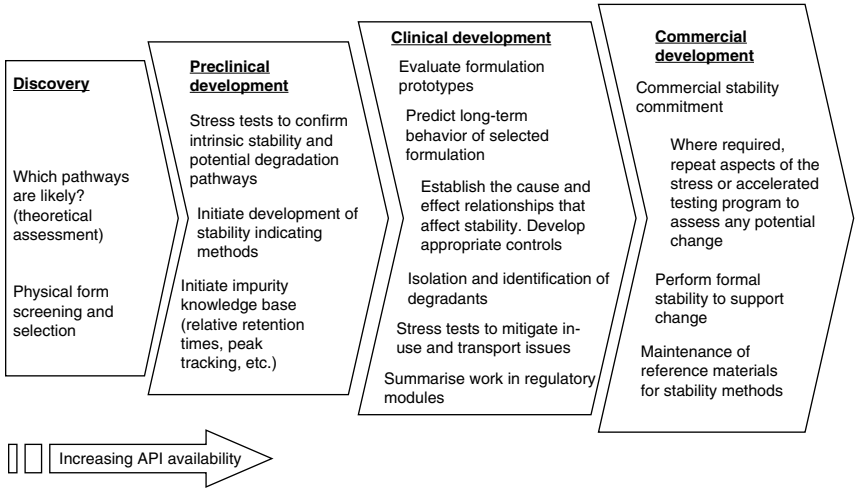

Discovery
Which pathways are likely? (theoretical assessment)
Physical form screening and selection
Preclinical development
Stress tests to confirm intrinsic stability and potential degradation pathways
Initiate development of stability indicating methods
Initiate impurity knowledge base (relative retention times, peak tracking, etc.)
Clinical development
Evaluate formulation prototypes
Predict long-term behavior of selected formulation
Establish the cause and effect relationships that affect stability. Develop appropriate controls
Isolation and identification of degradants
Stress tests to mitigate in-use and transport issues
Summarise work in regulatory modules
Commercial development
Commercial stability commitment
Where required, repeat aspects of the stress or accelerated testing program to assess any potential change
Perform formal stability to support change
Maintenance of reference materials for stability methods
Increasing API availability

Figure 1.8 Aspects of stability testing during the development lifecycle.

differences are observed, these are discussed with the relevant regulatory authority and where required additional protocols are executed that align with the different regional requirements.

1.3.4 Conclusions

The ICH stability guidelines provide the core framework for the stability testing intended for the registration of medicines in the ICH regions. As the guidance is derived from thermodynamic and kinetic principles, it can also be flexibly applied during the early development phase, to generate supporting knowledge. The different aspects of stability testing performed during development are summarized in Figure 1.8. The guidance accommodates alternative approaches where justified and also informs stability guidance beyond the ICH regions. All of these aspects ensure that the stability guidelines are widely applied globally, either in total or with some specific differences recommended in certain territories.

References

1 ICH Q1A(R2) (2003) Stability Testing of New Drug Substances and Products (Revised Guideline).
2 ICH Q1C (1996) Stability Testing of New Dosage Forms.
3 World Health Organization, WHO Technical Report Series, No. 953, 2009 Annex 2 Stability testing of active pharmaceutical ingredients and finished pharmaceutical products.
4 ICH Q8 (R2) (2009) Pharmaceutical Development (Revised Guideline).
5 ICH Q9 (2006) Quality Risk Management.
6 ICH Q10 (2008) Pharmaceutical Quality System.
7 ASEAN guideline on stability study of drug product (May 2013), Version 6.
8 TITLE 21—Food And Drugs Chapter I—Food and Drug Administration Department of Health & Human Services Subchapter C—Drugs: General Part 211—Current Good Manufacturing Practice for Finished Pharmaceuticals Subpart G—Packaging and Labeling Control Sec. 211.137 Expiration Dating.
9 Parenty ADC, Button WG, and Ott MA (2013) An Expert System to Predict the Forced Degradation of Organic Molecules, *Molecular Pharmaceutics*, 10(8), p 2962–2974.
10 Waterman KC, Adami RC, Alsante KM et al. (2002) Hydrolysis in Pharmaceutical Formulations, *Pharmaceutical Development and Technology*, 7(2), p 113–146.
11 Horvoka SW and Neich CS (2001) Oxidative Degradation of Pharmaceuticals: Theory, Mechanisms and Inhibition, *Journal of Pharmaceutical Sciences*, 90, p 253–269.

12 Turro NJ (1991) *Modern Molecular Photochemistry*, University Science Books: Sausalito, CA.

13 Garcia C, Cabezas K, Nonell S et al. (2014) Substituent Effect on the Photostability of 4-Aryl-1,4-Dihydropyridines, *Photochemistry and Photobiology*, 90, p 73–78.

14 Stewart PJ and Tucker IG (1985) Prediction of Drug Stability—Part 4: Isomerisation, *The Australian Journal of Pharmacy*, 15(3), p 181–188.

15 Qiu Z, Stowell JG, Cao W et al. (2005) Effect of Milling and Compression on the Solid-State Maillard Reaction, *Journal of Pharmaceutical Sciences*, 94(11), p 2568–2580.

16 Crowley P and Martini L (2001) Drug-Excipient Interactions, *Pharmaceutical Technology Europe*, 13, p 26–34.

17 Narang AS, Divyakant D, and Badawy S (2012) Impact of Excipient Interactions on Solid Dosage Form Stability, *Pharmaceutical Research*, 29, p 2660–2683.

18 Yoshioka S and Aso Y (2007) Correlations Between Molecular Mobility and Chemical Stability of Amorphous Pharmaceuticals During Storage, *Journal of Pharmaceutical Sciences*, 96, p 960–981.

19 Grimm W (1985 & 1986) Storage Conditions for Stability Testing—Long Term Testing and Stress Tests, *Drugs Made in Germany*, 28, p 196–202, 1985 and 29, p 39–47, 1986.

20 Schumacher P (1974) Aktuelle Fragen zur Haltbarkeit von Arzneimitteln, *Pharmazeutische Zeitung*, 1(19), p 321–324.

21 Ammann C (2011) Stability Studies Needed to Define the Handling and Transport Conditions of Sensitive Pharmaceutical or Biotechnological Products, *AAPS PharmSciTech*, 12(4), p 1264–1275.

22 European Commission Guideline (2013) Good Distribution Practice of Medicinal Products for Human Use, 2013/C 343/01.

23 Waterman KC and Macdonald BC (2010) Package Selection for Moisture Protection for Solid, Oral Drug Products, *Journal of Pharmaceutical Sciences*, 99(11), p 4437–4452.

24 Waterman KC (2011) The Application of the Accelerated Stability Assessment Program (ASAP) to Quality by Design (QbD) for Drug Product Stability, *AAPS PharmSciTech*, 12(3), p 932–937.

25 Remmelgas J, Simonoutti AL, Ronquist Å et al. (2013) A Mechanistic Model of In-Use Moisture Uptake by Packaged Dosage Forms, *International Journal of Pharmaceutics*, 441, p 316–322.

26 Ahlneck C and Zografi G (1990) The Molecular Basis of Moisture Effects on the Physical and Chemical Stability in the Solid State, *International Journal of Pharmaceutics*, 62, p 87–95.

27 Byrn SR, Xu W, and Newman AW (2001) Chemical Reactivity in Solid-State Pharmaceuticals: Formulation Implications, *Advanced Drug Delivery Reviews*, 48, p 115–136.

28 Carstensen, JT (1977) *Stability and Dating of Solid Dosage Forms in Pharmaceutics of Solids and Solid Dosage Forms*, Wiley-Interscience, New York, p 182–185.

29 MacFaul PA, Ruston L, and Wood MJ (2011) Activation Energies for the Decomposition of Pharmaceuticals and Their Application to Predicting Hydrolytic Stability in Drug Discovery, *Medicinal Chemistry Communications*, 2, p 140–142.

30 Conners KA, Amidon GL, and Stella VJ (1986) *Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists*, 2nd Edition, John Wiley & Sons, Inc., New York.

31 Anderson G and Scott M (1991) Determination of Product Shelf Life and Activation Energy for Five Drugs of Abuse, *Clinical Chemistry*, 37(3), p 396–402.

32 Huang L-F and Tong F-Q (2004) Impact of Solid State Properties on Developability Assessment of Drug Candidates, *Advanced Drug Delivery Reviews*, 56(3), p 321–334.

33 Merritt JM, Viswanath SK, and Stephenson GA (2013) Implementing Quality by Design in Pharmaceutical Salt Selection: A Modeling Approach to Understanding Disproportionation, *Pharmaceutical Research*, 30, p 203–217.

34 ICH Q6A (1999) Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances.

35 ICH Q3A (R2) (2006) Impurities in New Drug Substances (Revised Guideline).

36 ICH Q3B (R2) (2006) Impurities in New Drug Products (Revised Guideline).

37 ICH Q3C (R6) (2016) Impurities: Guideline for Residual Solvent (Revised Guideline).

38 ICH Q3D (2014) Guideline for Elemental Impurities.

39 Ermer J (2004) Analytical Validation with a Special Focus on Stability Considerations, *American Pharmaceutical Outsourcing*, 5(1), p 24–32.

40 USP Validation and Verification Expert Panel (2013) Lifecycle Management of Analytical Procedures: Method Development, Procedure Performance Qualification, and Procedure Performance Verification, *Pharmacopoeial Forum*, 39(3), p 15–31.

41 Seevers RH and Schantz-Shirley R (2011) Regulatory Strategy for Long-Term Stability Conditions to Support Submission in Zone IV Countries, *Pharmaceutical Outsourcing*, 12(2), p 44–51.

42 Alsante K, Ando A, Brown R et al. (2007) The Role of Degradant in Active Pharmaceutical Ingredients and Drug Products, *Advanced Drug Delivery Reviews*, 59, p 29–37.

43 Baertschi SW (2005) *Stress Testing: A predictive Tool in Stress Testing; Predicting Drug Degradation*, Taylor and Francis, Boca Raton, p 13–49.

44 Kleinman MH, Elder D, Teasdale A et al. (2015) Strategies to Address Mutagenic Impurities Derived from Degradation in Drug Substances and

Drug Products, *Organic Process Research and Development*, 19(11), p 1447–1457.

45 Draft General Guidance on "Hold-Time" Studies (2014), World Health Organization Working Document QAS/13.521/Rev 2.

46 Guidance for Industry, Container Closure Systems for Packaging (1999) Human Drugs and Biologics, CMC Documentation, U.S. Department of Health and Human Services, FDA Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER).

47 Note for guidance on in-use stability testing of human medicinal products (2001), CPMP/QWP/2934/099.

48 Acken B, Alessandro M, Colgan ST et al. (2012) Industry Perspectives on GMP in Early Development—Part 4: Stability, *Pharmaceutical Technology*, 36(9), p 64–70.

49 Waterman KC, Adami RC, and Hong J (2003) Chapter 4 Impurities in Drug Products in *Handbook of Isolation and Characterization of Impurities in Pharmaceuticals*, Eds Ahuja S and Alsante KM, Academic Press, Amsterdam, p 75–87.

50 Li G, Schoneker D, Ulman KL et al. (2015) Elemental Impurities in Pharmaceutical Excipients, *Journal of Pharmaceutical Sciences* 104, p 4197–4206.

51 Salemeh AK and Taylor LS (2006) Physical stability of Crystal Hydrates and their Anhydrates in the Presence of Excipients, *Journal of Pharmaceutical Sciences*, 95(2), p 446–461.

52 Beaman J, Whitlock W, Wallace R et al. (2010) The Scientific Basis for the Duration of Stability Data Required at the Time of Submission, *Journal of Pharmaceutical Sciences*, 99, p 2538–2543.

53 ICH Q1E (2003) Evaluation of stability data.

54 ICH Q1D (2002) Bracketing and Matrixing Designs for Stability Testing of New Drug Substances and Products.

2

Stability Testing

Photostability Testing of New Drug Substances and Products ICH Q1B

David Clapham

David Clapham, Independent Pharmaceutical Consultant, Hertfordshire, UK

2.1 Introduction

The ICH Q1B photostability testing guideline [1] was recommended for adoption in November 1996 and was published by the FDA (Food and Drug Administration), MHWL, and EMEA between May 1997 and January 1998. It has not been revised since. It is an annex to the Stability Testing of New Drug Substances and Products guideline Q1A [2], and the two should be read in conjunction with each other particularly with respect to deciding what constitutes an acceptable change, especially regarding the level of degradation products.

Exposure to light is almost certain for all pharmaceutical products, and the importance of photostability testing within the pharmaceutical industry is well recognized in several books [3, 4] and journal articles [5–7] dedicated to the subject and a range of training courses available.

Photons will be absorbed by most active pharmaceutical ingredients (APIs) and/or components of pharmaceutical formulations. The absorbed energy can often be dissipated harmlessly. However it can also lead to bond breakage leading to degradation of the API and/or unacceptable changes to the formulation or delivery system [8]. Photodegradation can occur via photo-induced oxidation, hydrolysis, ring opening, isomerization, and polymerization, to name a few. In some cases several of these mechanisms may occur sequentially or simultaneously. Thus photochemistry can be complex. Light-induced changes in formulations include changes of color (either coloration or bleaching of added colors) [9], degradation of stabilizing polymers leading to viscosity changes/sedimentation [10], heat-related melting and embrittlement of packs and capsule shells [11], tablet hardness changes, etc. [12].

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Hence photostress testing is an important component of the drug development process, and investigators both in academia and industry have investigated this area for many years [13–16]. These studies involved a wide variety of experimental practices including different photolytic sources, spectral ranges, exposures, and protocols. The guideline helps to clarify and standardize light radiation sources and the amounts of exposure needed to assess the photostability of drug substances and drug products. However, as the large number of published commentaries attest [17–20], there is still significant room for additional guidance on how to interpret the guidance and how to practically perform a photostability test. It is also important to be clear about what the guidance does *not* say, which could nevertheless be important in gaining an understanding of the effect of light on a particular drug substance and formulation.

This chapter will firstly cover what the guidance requires and provide some help in translating this into an experimental protocol. It will then consider additional factors that should be considered in order to obtain a thorough scientific understanding of the product and how it should be handled in practice.

2.2 What the Guidance Says

The guideline aims to cover two very different types of test, namely, "forced degradation" and "confirmatory" studies.

Forced degradation testing is undertaken to purposely degrade the sample. It is clearly necessary to perform a forced degradation study in order to develop a suitable analytical method capable of visualizing the potential photodegradants (or demonstrating the specificity of an existing method) prior to undertaking a confirmatory test. Forced degradation studies are also used to elucidate degradation pathways and guide controls needed for the manufacture, packaging, storage, and use of API and drug product.

There is limited guidance in the Q1B document concerning these specific studies. In particular the amount of light exposure required is not spelt out. For photolabile compounds reference can be made to other ICH guidance and good practice for other types of forced degradation also applies. Thus the conditions should be chosen to achieve approximately 10–20% overall degradation [21] (or less where scientifically justified) in order to ensure that the primary photodegradation products are observed. In order to understand the kinetics of the process, it is wise to include intermediate time points as well. The major difficulty here is to decide how to handle a photostable sample and in particular how much light exposure to subject the sample to. Some studies have used up to 10-fold the confirmatory study exposure levels. However the emerging consensus is to employ no more than twice the confirmatory study levels

(which approximates to 24 h in a Suntest CPS+ solar simulator™). Keeping the light dose to approximately 2xICH exposure levels is practical as it ensures efficient use of the light stability chamber and reduces the likelihood of secondary photoproducts. If a sample has not shown significant degradation within this period, it is unlikely to do so with further exposure [22]. Furthermore, the light exposure for a confirmatory study reflects what is thought to be roughly equivalent to 3 months of continuous exposure to typical ultraviolet (UV) and visible light without protective packaging in a pharmacy, warehouse, or home in the United States/northern Europe. Obviously for geographical locations with more hours of sunshine and/or higher intensity, then this period would be considerably shorter. Even so, the photostress tests are doubling this "worst-case scenario" and any further extension of the study is likely to represent an unrealistic situation.

So what of the confirmatory test itself? This is the test that has the most detailed guidance in the document and as the name suggests is designed to confirm that the pack is appropriate for the protection of the API and drug product. These studies are similar in concept to the accelerated or formal stability studies described in the parent guideline Q1A, and it is this test that many people are thinking of when they refer to an ICH photostability study. The confirmatory test utilizes fixed levels of light exposure. For a photolabile drug substance or drug product outside its protective pack, the required exposure may be harsher than would be required for a forced degradation study. Conversely if a photostable drug product is exposed to the forced degradation, study conditions described above it could be possible to make the scientific argument that there is no need to repeat the confirmatory study, provided that the material tested is representative. It is important to remember that the confirmatory study is conducted not only on the API but also on the formulation. As discussed in the succeeding text, there are examples in the literature of photostable API exhibiting instability in a formulation. Of course formulation approaches (as well as packaging options) can often be used to stabilize a photounstable API, and this will also be briefly discussed later.

The Q1B guidance provides a stepwise protocol for testing that is summarized in the following flowchart (Figure 2.1).

For API there could be an additional step prior to the commencement of testing (though drug product would still need to be tested). According to the first law of photochemistry, light absorption is *required* for photoreactivity [23]. Thus if the molecule does not absorb light above 290 nm (the lowest wavelength that is present in the sunlight at the Earth's surface—see Figure 2.2), then the molecule is intrinsically photostable. If even a low level of absorption is seen, then the test should be performed. A "cutoff" for the permissible level of absorption can be derived from the phototoxicity testing guideline ICH S10 [24]. If a compound does not have a molar extinction coefficient (MEC) greater than 1000 l/mol/cm at any wavelength between 290 and 700 nm, it is unlikely to

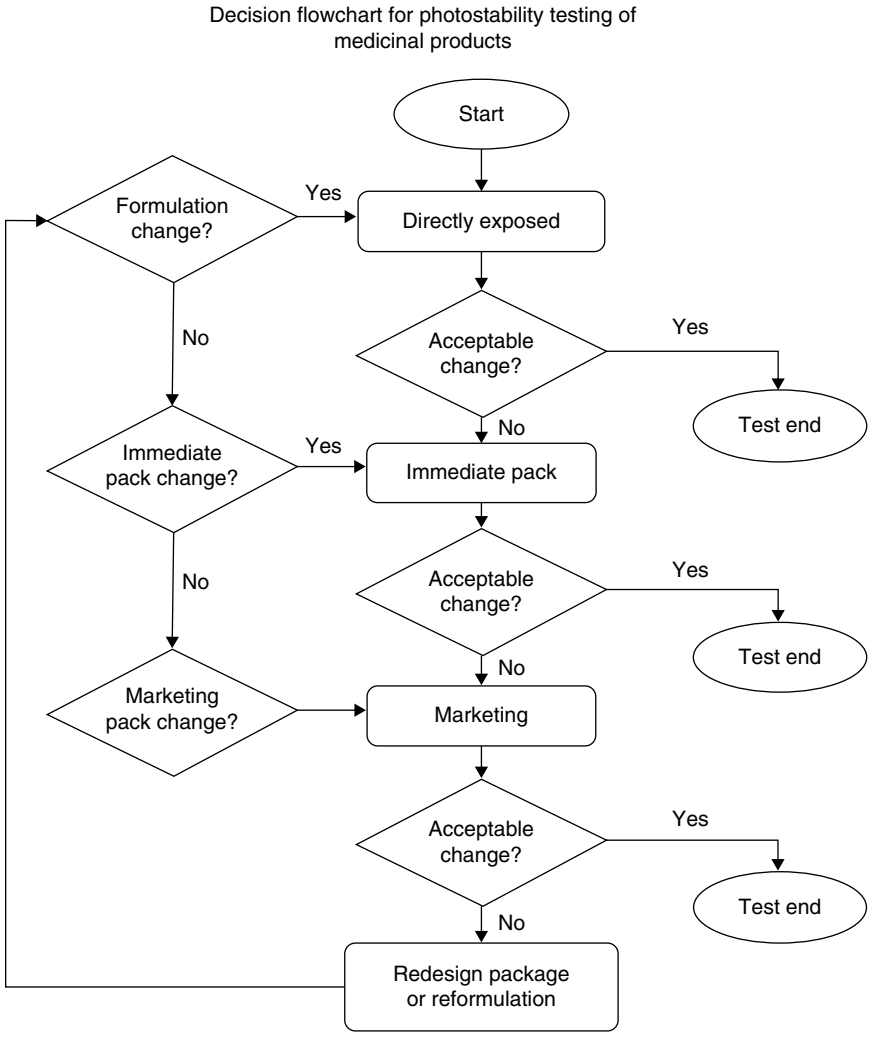


Figure 2.1 ICH Q1B flowchart.

be sufficiently photoreactive to result either in significant photodegradation or in direct phototoxicity. Phototoxicity testing is another important aspect of the overall assessment of the interaction of light with a pharmaceutical and will be discussed in a little more detail later in this chapter (see Section 2.4.7).

The next step is to expose the sample to a *minimum* of 120 million lux hours of visible light and 200 W/m^2 of integrated UV light either unprotected, in the immediate pack or in the marketing pack and to evaluate the sample for acceptable change under these exposure conditions. The guideline provides some

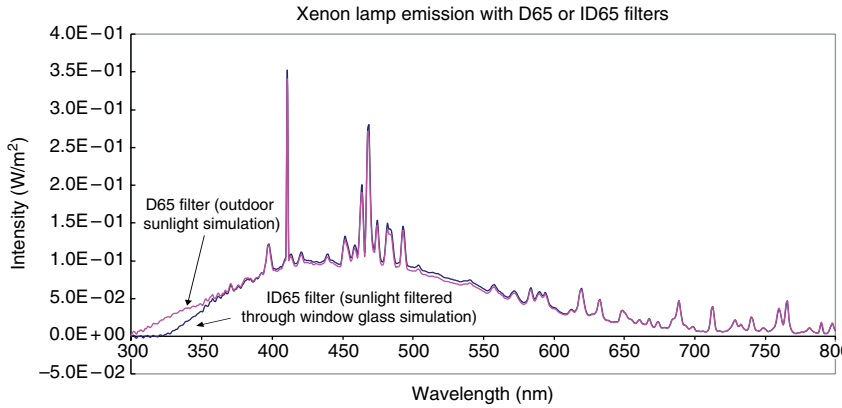


Figure 2.2 Spectral power distribution of a xenon lamp that simulates outdoor or window-filtered sunlight.

guidance on the light sources to use for the test in two groups, namely, Option 1 and Option 2 (see *How do I provide the required level of light?*). It is at this point that a lot of the questions arise on how to actually undertake such a study.

These studies may be conducted at any stage of development. If the product requires photo protection, then it is obviously wise to gain information early so that appropriate formulation and packaging approaches can be built into the development strategy. It may even be possible to use chemical SAR knowledge to design a molecule that does not have a photostability issue. The guidance directs that photostability testing should be conducted on at least one batch of drug substance and drug product (preferably using representative materials including excipients and processes). If clear-cut results are obtained (that the compound is either photostable or photolabile), they must be confirmed on one batch of each at production scale. If they are not clear-cut, it is then necessary to confirm on a further two batches, and final conclusions must then be based on a sound scientific rationale probably including a "weight of evidence" assessment.

2.3 What the Guidance Does Not Say

In this section we will consider the practicalities of conducting an ICH compliant photostability confirmatory test and what the guidance either does not say or does not make fully clear. This will be based on providing answers to some frequently asked questions. This additional guidance is not proscriptive. There are many ways of conducting an ICH-compliant photostability test. Any approach that is scientifically justified may be used. However this discussion should prove helpful in the majority of cases.

Question 1. Are the ICH Tests All I Need to Do?

The objective of photostability testing, as with any stability testing, is to ensure that the product reaches the patient in a form that will have an optimum benefit/risk profile. The applicant needs to be able to demonstrate a thorough scientific understanding of their drug product to a regulator, and hence the answer to this question will depend on the initial results. For a photolabile drug substance/product, it may well be necessary to conduct additional photostability studies above and beyond those described in Q1B in order to gain a thorough understanding of the photostability. Thus ICH Q1B studies are necessary but may not be sufficient in and of themselves (see Section 2.4).

Question 2. What Is Acceptable Change?

It is up to the development scientist to define this. The Q1B guidance makes the open-ended statement that "Acceptable change is change within limits justified by the applicant." In terms of the chemical changes, the acceptable level of impurities/degradants, and the need to identify and/or qualify them, the parent Q1A guidance along with that in ICH Q3A [25] and ICH Q3B [26] holds. However, as noted previously light can induce a number of other changes in either API, drug product, or both. In some cases these changes will be more significant in terms of product quality than the chemical changes. Any change that adversely affects quality, safety, and/or efficacy is of course unacceptable and remedial action would be required.

Some obvious unacceptable changes (or at least changes that will require further investigation) include

- Loss of active with concomitant loss in efficacy
- Changes that can affect bioavailability (e.g., change of form, isomer)
- Formation of toxic degradants
- Formation of stable products with different pharmacological activity than the parent drug
- Physical changes that are perceptible to the patient
- Changes that reduce the ability to reproducibly manufacture the API or DP

Table 2.1 provides guidance of some factors that should be assessed.

Question 3. How Do I Provide the Required Level of Light?

The guidance describes two possibilities here:

- Option 1—A single lamp that provides both UV and visible light with an output *similar to* D65/ID65 [27] emission standard. The required degree of similarity to this standard is not defined. Options include artificial daylight fluorescent lamp combining Vis/UV light, xenon arc lamps, and metal halide sources. Most formulations (with the possible exception of topical

Table 2.1 Additional tests to consider when evaluating photosensitivity.

		Drug products	
Test	**Drug substance**	**Sterile/liquids**	**Oral solid**
Appearance	✓	✓	✓
Impurity/potency assay	✓	✓	✓
Chirality	✓	✓	✓
Color	✓	✓	✓
Particulates (nephelos/HIAC)	✓	✓	
Preservatives		✓	✓
Flavorings/functional excipients		✓	✓
pH		✓	
Osmolality		✓	
Packaging changes		✓	✓
Re-suspendability		✓	
Reconstitution time		✓	
Viscosity		✓	
Hardness			✓
Friability/disintegration			✓
Dissolution			✓

formulations) will not be exposed to direct outdoor sunlight (D65) for a significant length of time during manufacture, shipping, handling, storage, or use, and hence it is usually appropriate to use an equivalent of the ID65 standard. For high intensity sources this can be achieved by using a filter between the lamp and the sample chamber.

- Option 2—Here, the same sample is exposed to both a cool white fluorescent and a near-UV lamp either simultaneously (all lamps are "on" concurrently) or in series (often in different light chambers).

Each has its advantages and disadvantages, some of which are summarized in Table 2.2. The guidance does *not* imply that these two options are equivalent.

For example, it is not possible to provide the exact levels of UV and visible light exposures called for in the guidance with an Option 1 source. These sources provide an overabundance of UV light relative to visible light, so exposure to the required 1.2 million lux hours will provide significantly more than the required 200 Wh/m^2 of integrated UV light. However these sources provide a good simulation of sunlight and can therefore be considered to be representative of "real-world" exposure.

Table 2.2 Advantages and disadvantages of Option 1 and Option 2.

Practical issue	Comment	Rationale
Heat	Option 1 exposes samples to more heat than Option 2	Option 1 lamp sources are more intense and create more heat. Option 1 usually also requires a cooling unit that may provide an issue for sample presentation due to blowing of air
Exposure time	Option 1 requires less time for irradiation	Typically, Option 2 is conducted sequentially and with less intense lamps
Spectral gap	Option 2 features multiple lamps that have poor overlap or poor output between 380 and 400 nm	Option 1 has a single lamp with broad spectral distribution
Overexposure	Use of Option 1 typically results in overexposure of UV component	Lamps used for Option 1 (e.g., xenon) emit a large amount of UV, so it takes longer for the sample to be exposed to the required level of visible light
Spectral range	Option 1 mimics outdoor daylight; Option 2 mimics indoor light	Refer to ISO standards

Exact exposures are possible with Option 2 sources as the lamps can be switched on/off independently. However this is not representative of what the sample is likely to be exposed to unless it is exposed solely to indoor light (where the UV exposure may well be significantly less than that required by the guideline). The near-UV fluorescent lamp has a spectral distribution from 320 to 400 nm with a maximum energy emission between 350 and 370 nm. A significant proportion of UV should be in both bands of 320–360 nm and 360–400 nm. However there is an emission gap between the two sources (380–420 nm) where there is little output from either lamp, so caution should be taken if the API absorbs strongly in this region [28].

Some further factors to consider when using Option 2 are as follows:

a) The order of exposure of the sample to the UV and visible light lamps (if used separately) may be important. For example, it is distinctly possible that exposure to the UV lamp first will produce a colored degradation product that will then absorb visible light, potentially leading to further degradation. This would not occur if the visible exposure occurred first, potentially providing a different outcome from the experiment.
b) The output from a fluorescent lamp tends to be more "spiky" than that from a solar simulator (Figure 2.3). If the compound/formulation has an absorption maximum at the wavelength of one of these spikes or if such a wavelength is important in the degradation process (see Section 2.4.3.1), then a

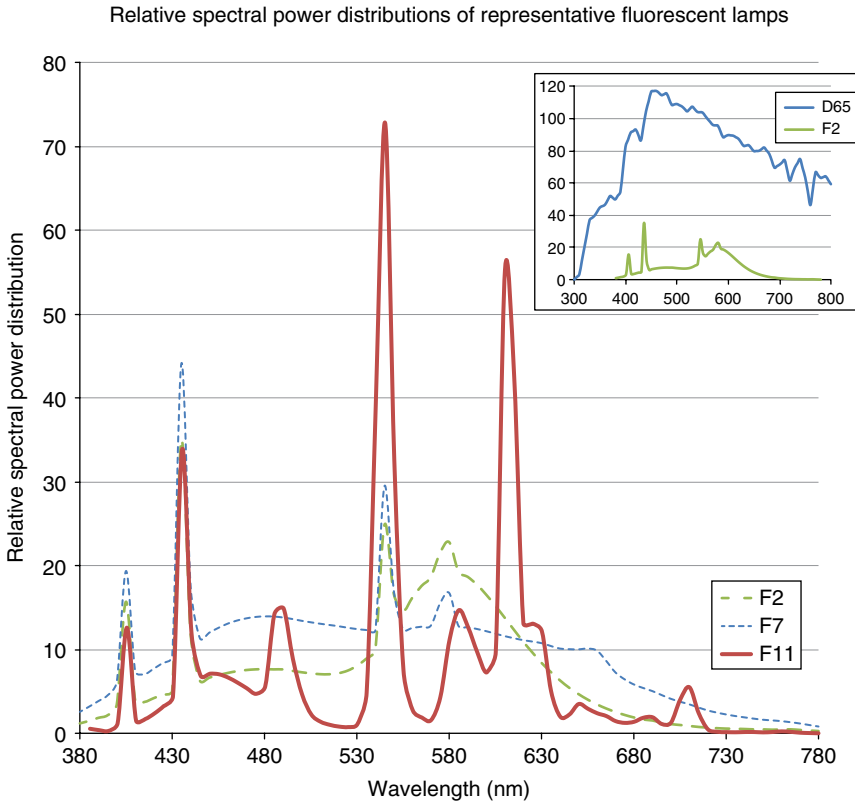


Figure 2.3 Relative spectral power distributions of various representative fluorescent lamps plotted from published CIE data. F2 represents a cool white fluorescent source that is compliant with ICH Q1B Option 2; F7 represents a typical "broadband" fluorescent source; and F11 represents a triphosphor or three-band cool white fluorescent source. The inset shows the spectral power distribution of one source (F2) relative to daylight (D65).

fluorescent lamp may prove more detrimental to the API or formulation even though the overall light power would appear to be lower.

c) The experimental time is much longer for an Option 2 source than an Option 1 source due to the lower power of the lamps. A typical exposure time to deliver the confirmatory test requirements is 5 days (120 h for a 1000 lux lamp) for the visible component and 1–2 days for the UV component, whereas it is between 7.5 (750 W/m^2) and 22 h (250 W/m^2) based on the visible light requirement for a xenon arc (Option 1) source.

The choice of which option to use should therefore depend on the scientific question being asked. For a full understanding of the photostability behavior of an API or DP, it may be necessary to use both ICH options of light sources.

Question 4. How Do I Know if I Have Provided the Right Amount of Light?

The good news here is that the guidance says that you can rely on the manufacturers' information for the light source/cabinet that you use and calculate the exposures based on time. Of course normal qualification and validation procedures should be applied. In order to allow for variability, it is wise to apply a small overage to the calculated time (typically 1.2 times the longest time) since the requirement of the guideline is that the sample receives a *minimum* of 1.2 million lux hours and a *minimum* of 200 W/m^2 integrated UV light. However given the potential for lamps to change with time and for nonuniformity within a light cabinet (see succeeding text), there may be value in measuring the amount of light a sample actually receives.

The guidelines provide a method of assessing the UV dose given using a chemical actinometer. It is important to realize that this measures only the UV component and does not measure the visible component. The actinometer suggested is based on quinine, and there is a significant body of literature based around the issues associated with using this actinometer [29, 30] and often proposing simpler alternatives [31]. Suffice it to say here that reliable results can only be obtained from actinometry if the behavior of the actinometer is fully understood and the required handling precautions are taken. There are a range of physical methods of measuring light (see Table 2.3) and literature around using each. An excellent free book is available from International Light Technologies on the whole topic of measuring light [32].

Question 5. In What Form Should I Present My Sample?

The answer to this question again depends on the scientific question being asked.

In general, the photosensitivity of drug substances or products follows the order solutions > suspensions/dispersions >> crystalline solids. Solid formulations that contain the drug in an amorphous form, for example, lyophiles, spray-dried powders, solid solutions, solid dispersions, etc., may have greater photosensitivity than a crystalline version of the same compound [33]. The polymorphic form can also have an influence on photostability, while exposure to light can influence polymorphic form [34, 35]. Hence it is important to use representative material for the test.

All compounds should be assessed as solids since this is how they will normally be stored. In early stage development it may well be advisable to test a solution of the API in order to understand the photochemistry that could occur no matter what the final intended formulation; though of course the interpretation of the impact of any photodegradation seen needs to take the intended formulation into account. Testing of a solution/suspension is of course mandatory if this is the final dosage form (e.g., an injection) and also as part of the

Table 2.3 Methods of measuring light power.

Methodology	Notes
Actinometry	No specialist equipment required
	Many versions available
	Amount of light (generally UV) determined via a chemical change
	Requires thorough understanding of the photochemistry involved and careful handling precautions to obtain valid results
Lux meter	Measures visible light only
	Is generally calibrated to the photopic curve and hence not strictly valid for intense light sources
UV meter	Measures UV portion only
	Ensures choice of correct wavelength range and correct
	power range for valid calibration
Optical power meter	Requires correct choice of sensor and mode
	Provides an overall power reading but no information about the power distribution. Need to understand sensor response profile to be able to calculate UV and visible portions. Calibrate with the light source
Spectroradiometer	When properly calibrated with an appropriate attenuation for the power of the light, source can provide both power and spectral power distribution data Relatively expensive

forced degradation study. This then leads to some further questions. For example

a) Is there a preferred cosolvent if one is needed to achieve a solution? Opinion is split on this since the solvents themselves can engage in photochemistry and/or form adducts with the compound under test. A common (but by no means universal) cosolvent is acetonitrile. Whichever solvent is used, it is important to use it at the lowest concentration necessary to achieve the required solubility. Having said that one method of assessing the contribution of the solvent to the overall photochemistry is to repeat the assessment with solutions containing different levels of cosolvent, it may also be wise to compare results using different cosolvents. An alternative to using a cosolvent is to use a more dilute solution (or to accept the need to assess a suspension).
b) Is there a recommended concentration of sample? For an early stability study, the solution concentration may well be dictated by the availability of compound. A typical concentration is 100 μg/ml. The effect of concentration on photoreactivity follows the standard physical laws (e.g., the Beer–Lambert law), and these should be considered when interpreting results.

Absolute levels of degradants (within ICH Q1A guidance) should be considered as well as the percentage degradation particularly if the degradants are known to be toxic or genotoxic. When assessing a suspension the particle size will be important since this will change the surface area available to react to the light, while the concentration will be important since it will influence the light penetration through the sample/reflection by the sample.

c) Is particle size important? The short answer is yes. The magnitude of the effect depends on the size range and the photochemical reaction involved [36].

Finally, photochemical reactions may not always be the same in the solution and solid states. As a general rule, reactions in solution simply occur faster than those in the solid state; however it is entirely possible for the mechanism to differ too. An example is provided by menadione that undergoes [2+2] dimerization in the solid state but in ethanol solution yields an epoxide as the major product [37]. Another is that cyclodecanedione undergoes a novel photorearrangement in the crystalline state that is different from the Norrish/Yang type II photocyclization process observed in solution [38].

The "take-home" message is that the analyst should present the sample in a form that is representative of the final API and drug product. Additional studies may also be valuable to aid understanding of the photostability and hence the likely effect of any changes that are being considered. Of course the accuracy of any predictions will need to be confirmed.

Question 6: In What Way Should I Present My Sample?

Sample presentation is a very important practical consideration for photostability testing. The main issues to consider are containment, quantity, and positioning.

Since all lamps produce heat as a by-product and one mechanism of dissipating absorbed light is as heat, many photostability cabinets include powerful fans to aid cooling. Thus all samples will need to be contained for both practical and safety reasons. In the majority of the cases, the sample will be in a pack and so will be contained to some degree—but it is important to ensure that the closure of that container is sufficient to resist any pressure change within the container caused by a temperature rise. It must also not lose integrity due to the effect of light on the pack itself. For "open" exposure studies the obvious way forward is to contain solutions in quartz cells, while for powders they can be exposed in a dish covered with a quartz glass plate. There are some practical difficulties here. For example, a closed system may not allow absorbed heat to escape, potentially leading to thermal degradation as well as photolytic change. There may also be a need for additional sample handling for analysis. Work

within our laboratories suggests that for solutions the attenuation due to thin-walled type 1 glass vials is insignificant, allowing exposure in HPLC vials ready for analysis. For solids, covering the sample with a thin plastic film such as food wrap (e.g., cling film) with or without small holes to allow gas transfer can give useful results.

Even with good cooling there may still be some temperature rise in the sample, and so it is important to also expose a sample wrapped in aluminum foil alongside the test sample. This control sample will allow the photolytic and thermal contributions to be deconvoluted. Of course during storage photolytic degradation is likely to be additive with degradation from all other sources, and this needs to be considered when assigning a shelf life [39].

Figures 2.4, 2.5, and 2.6 provide insights into the questions of quantity and positioning.

For a solid sample light will only penetrate the upper layer (Figure 2.4), the depth of penetration being somewhat dependent on the particle size [40]. Thus a thin film (typically 1–2 mm) should be exposed. When considering a monolithic solid dosage form such as a tablet, the surface that is directed toward the light source may show significantly greater change than the other face of the tablet that is in effect shaded by the bulk. This can lead to changes in appearance and changes to purity for that face only. These will not be evident if the whole tablet is homogenized and assayed since levels of degradants are likely to be small. Thus the scientist should consider any impact of changes to that face alone on the overall acceptability of change. Factors to be considered include likely patient perception of quality based on, for example, appearance changes as well as potential toxicology of any degradants. For a blister pack it

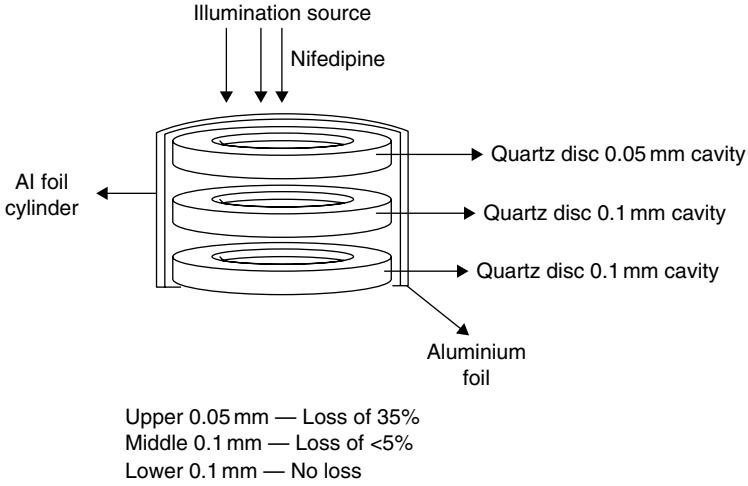

Illumination source
Nifedipine
Al foil cylinder
Quartz disc 0.05 mm cavity
Quartz disc 0.1 mm cavity
Quartz disc 0.1 mm cavity
Aluminium foil
Upper 0.05 mm — Loss of 35%
Middle 0.1 mm — Loss of <5%
Lower 0.1 mm — No loss

Figure 2.4 The effect of sample quantity.

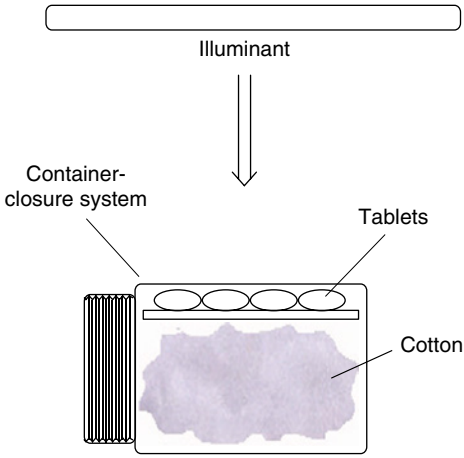


Figure 2.5 A possible pack to test a single layer of tablets in a pack.

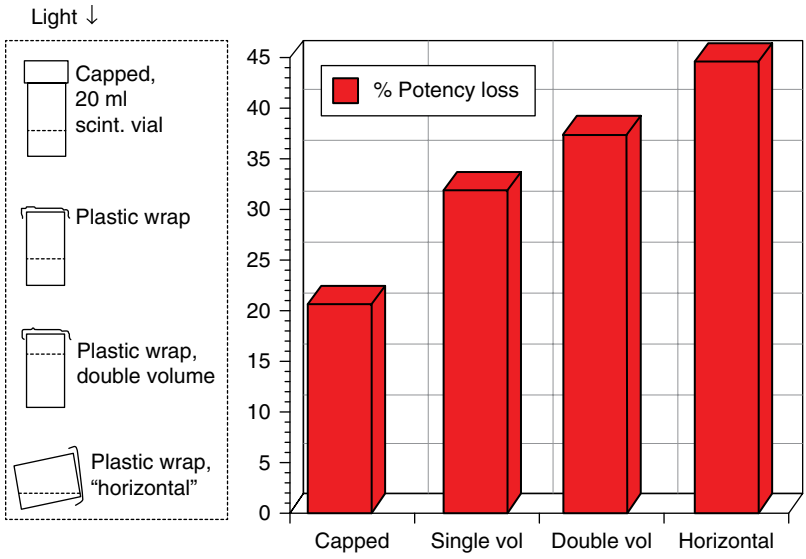


Figure 2.6 The effect of sample positioning.

is obvious that a single face will be more exposed to the light source. However this is also true to some extent in a bulk pack of those tablets closest to the pack wall. To allow this to be checked, an arrangement such as illustrated in Figure 2.5 might be considered [40]. It is of course important to ensure that the support material does not itself contribute to any reaction (e.g., some support materials could release formaldehyde). This also complies with the requirement in the guidance "… samples should be positioned to provide **maximum**

area of exposure to the light source. For example, tablets, capsules, should be spread in a **single layer** … Some adjustment of testing conditions may have to be made when testing large volume containers (e.g., dispensing packs)."

Figure 2.6 illustrates some factors to be considered when testing solution samples.

The capped sample shows the least degradation. This is because light is directional and the cap will shield the content. The maximum shielding will be directly perpendicular to the light source with increasing light entering the sample as the angle increases. Due to reflection the samples nearest to the chamber walls may well show the greatest degradation. This raises a general point. Shading/reflection can be caused by other samples in the vicinity of the sample under test depending on the nature of its container. As a "rule of thumb," samples should be separated by at least 1.5 times the height of the sample.

The effect of volume is illustrated by the second two bars. Light power varies as the square of the distance between the sample and the light source (the inverse square law). With a larger volume the surface is nearer the light source and experiences more photons per unit time. Generally the light power in a cabinet is measured at the floor. This should be considered when planning how to test a large sample such as a large volume parenteral. The final sample in this figure has the same volume as the first sample and illustrates that the amount of light absorbed is dependent on the surface area exposed to the light source. The appropriate positioning of the sample in the light cabinet relative to the light source will depend on how the formulation is likely to be used in practice.

Finally in this section the light distribution within the cabinet needs to be considered. Despite the best attempts of lighting engineers to compensate for the directional nature of light emission from the source and to even out exposure within a light cabinet, nevertheless light distribution within a cabinet will not be fully uniform. In some cases the difference in light power with position in a cabinet can be large. An example of a power map within a well-constructed cabinet is shown in Figure 2.7. It is important to have a clear understanding of the illumination power and the spectral power distribution at the point in the cabinet where the sample is placed. By extension, care should be taken when comparing results from samples placed at very different locations within a cabinet or in different cabinets. Similarly, sample chambers should not be overfilled; otherwise differences in results may be due to differential illumination rather than any other factor.

If samples are being used for comparative studies, they should be placed next to each other within the cabinet if possible; although it is generally acceptable to identify a region within the cabinet where the illumination level is *approximately* equal (e.g., ≥80% of the highest reading) and use this for all samples.

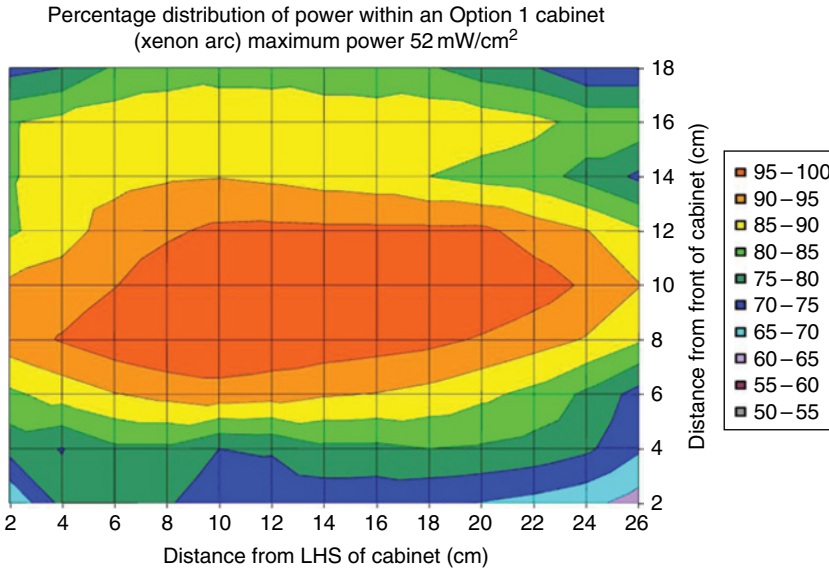


Figure 2.7 Power distribution within an Option 1 cabinet.

Question 7: Do I Have to Follow Each Step of the Flowchart (Figure 2.1)?

The answer depends on what you already know about the API and/or the formulation. It is permissible to use sound scientific judgment to avoid some of the earlier steps in the flowchart. For example, it may already be known that the compound is very photolabile (e.g., nifedipine), and it would therefore be rational to start at the immediate pack or marketing (secondary) pack stage. Following that approach has obvious implications for the required labeling of the product.

It is theoretically possible to provide a scientific argument for not undertaking a photostability test at all. As noted previously if the API and the formulation do not absorb light, they cannot photodegrade. Given the potential influence of synthetic route, polymorphic form, impurity profile, and formulation parameters, it is unlikely that an argument based on published data for the same chemical entity would be accepted as grounds for not performing the test. This would be particularly true if claiming that the published data proved the new version of the API was likely to be stable. If that published data confirms that the API and all formulations to date are photolabile, it would be rational to provide a scientific argument for only testing a packed product.

2.4 Other Factors to Consider

2.4.1 A Stable API Is Not Enough

Even an API that is stable can show degradation/physical changes in a formulation. Tapazole [41] does not absorb light above 290 nm but tablets show marked discoloration.

Similarly famotidine does not absorb light at any wavelength that overlaps with the solar spectrum, but when this API is formulated with riboflavin, significant photodegradation occurs. This is due to the generation of reactive oxygen species by the riboflavin on exposure to light, which can then degrade the famotidine [42].

As illustrated by Templeton et al. [43] some excipients can undergo Fenton chemistry [44] to produce reactive oxygen species that can then lead to compound degradation in an API that was photostable in the ICH test. One example is the reaction of citrate buffer with trace levels of iron in the presence of light to produce citrate and oxygen free radicals. In turn these can then produce oxidized/radical forms of the drug, leading to degradation and production of further oxygen radicals. Another example is the thermal degradation of glucose solutions, leading to the production of 5-Hydroxy methyl furfural (5-HMF) during autoclaving [50], which can then interact with light and oxygen to produce singlet oxygen that can degrade otherwise photostable compounds reconstituted with this vehicle. Thus it is vital (as directed in the guideline) to test both drug substance and drug products. If a change is made in the formulation, the assessment should be repeated.

2.4.2 A Photounstable API Is Not the End of the World

Fortunately there can be several methods of mitigating any photolability of an API. This includes choosing the right packaging such as enclosing the product in a lightproof secondary pack (e.g., a cardboard carton) or excluding light (or selected wavelengths) from the primary pack, for example, using an amber glass bottle for liquids, a cover for an infusion bag, or an opaque tablet bottle. More subtle variants include using colored film for blister packs or apparently clear film containing UV absorbers. Care should be taken when relying on the pack alone however for several reasons. One reason is that apparently opaque materials can still have significant transmission of certain wavelengths. If these happen to be causative of the degradation as described in the succeeding text, then the pack may not offer as much protection as it might at first appear. Another, probably more important, concern is that patients/professionals might remove the product from the pack. This might be done to improve visibility (e.g., to aid identification, check for particulates, display for sale, etc.), for

dosing convenience (e.g., in dosing aids), or during use [12]. It might therefore be desirable to use formulation approaches to stabilize the drug product. Typical approaches include using an opaque white film coat either with or without UV absorbing additives, colored film coats to absorb damaging wavelengths [45], inclusion of UV absorbers and/or radical scavengers in liquid/semi solid formulations, etc.

There is a significant literature on the topic of photostabilization that should be consulted for further guidance. Some example papers are given here [46, 47]; the pharmaceutical photostability books referenced in the introduction to this chapter also covers this topic.

2.4.3 Higher Illuminance May Give Different Results

Figure 2.8 [6] shows that the same total light power delivered at different illuminances (rate) can lead to a different extent of degradation. Thus faster experiments may not always be better—but this effect is rare, and the conclusion that molsidomine [6] is light sensitive would not change whichever experimental paradigm was adopted.

2.4.3.1 Causative Wavelengths

For some API/drug products, particular wavelengths present in the illumination source may be particularly important in causing unacceptable change.

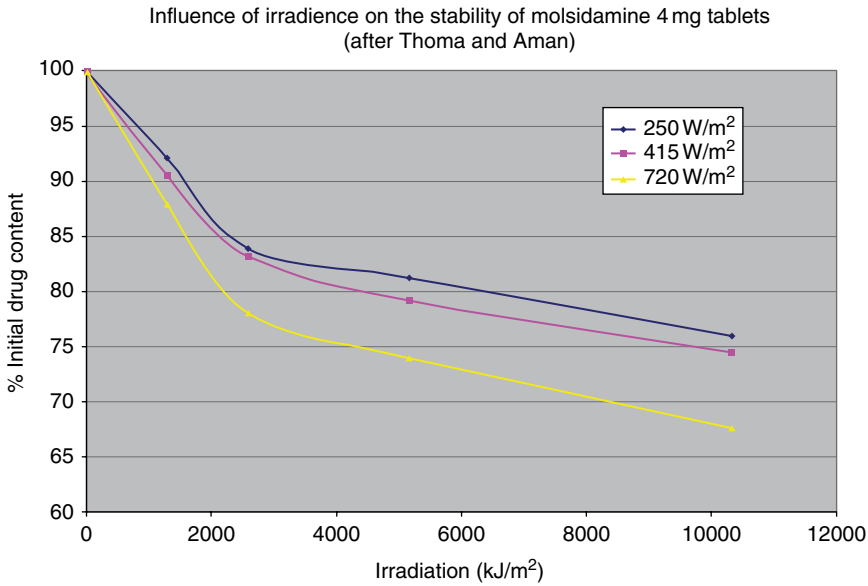


Figure 2.8 Impact of different illuminances (rate) on extent of degradation.

If this is so, then it stands to reason that exposure to a light source that happens to be particularly rich in those particular wavelengths will lead to more degradation for the same overall light power compared with a more uniform source. This is illustrated in Figure 2.9. The color of a sample can be measured using *L*, *a*, *b* tristimulus colorimetry [48]. The L value represents the lightness of a sample on an achromatic scale where 0 = black to 100 = white. The *a* value represents a red (+ve values)/green (–ve values) axis, while the *b* value represents a yellow/blue axis. The color of a sample can be unambiguously defined within the three-dimensional color sphere using these three values. In Figure 2.9 the loss of blue color from a tablet film coat (represented by less negative *b* values) is more marked under a fluorescent light source compared with a solar simulator for the same overall power. This is despite the fact that the fluorescent source is essentially devoid of any UV output compared with the significant UV component in the solar simulator. This effect is due to the fact that the fluorescent source is relatively rich in visible wavelengths that lead to the

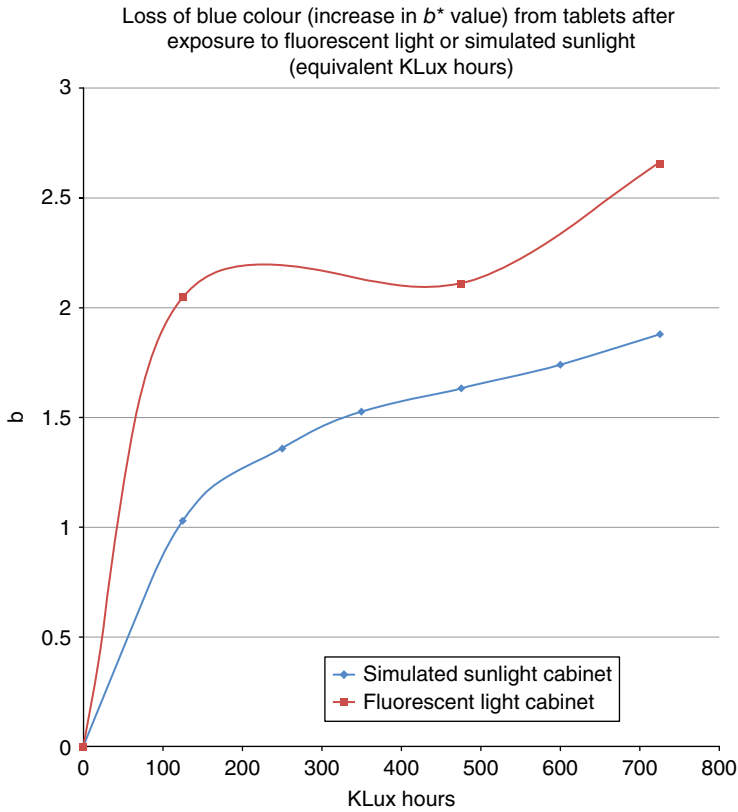
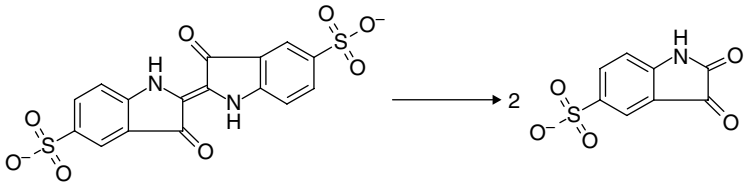


Figure 2.9 Effect of light source.

photolysis of the dye used in the coat. This may be important in the "in-use" setting if the sample is expected to be exposed to a light source that is significantly different to either Option 1 or Option 2 in spectral power distribution.

2.4.4 The Effect of Humidity

Many reactions are facilitated or accelerated by the presence of moisture. This includes photoreactions [49]. Given the high temperatures sometimes generated in light cabinets and/or high flow rates of air, the humidity with a cabinet can be quite low. Figure 2.10 illustrates this point where the color fade of a blue dye by light is accelerated in the presence of moisture compared with the "native" conditions within a xenon arc solar simulator cabinet. In this case the reaction concerned is the photocleavage of blue indigo carmine to colorless 5-isatinsulfonate, but photohydrolysis and photooxidation are both enhanced by the presence of moisture.



Indigo carmine 5-Isatinsulfonate

To assess this effect samples may be exposed with a small dish containing water or saturated salt solutions (controlled relative humidity) in the same container as the solid sample under test. Humidity is of course present to varying degrees in the storage environment.

2.4.5 Monitoring Chemical Change Alone Is Not Enough

As briefly touched on elsewhere in this chapter, light can induce many more changes in an API or DP than simple chemical degradation. Determination of "acceptable change" should encompass evaluation of all aspects that may have impact on a patient. Given the multifactorial nature of potential changes, this section cannot be definitive but factors to consider include:

- Temperature changes (Figure 2.11) that show that a blue sample can be up to 12°C hotter than a white one. This temperature change can then lead to melting, cracking of packs, and thermal degradation (among others).
- Appearance changes—Light exposure can lead to the generation of colored degradation products that may have a strong sensory impact even when present at levels that are hard to detect chemically in terms of measurable impurities. On the other hand it may cause bleaching of added color with potential for different strengths of a single product or even different

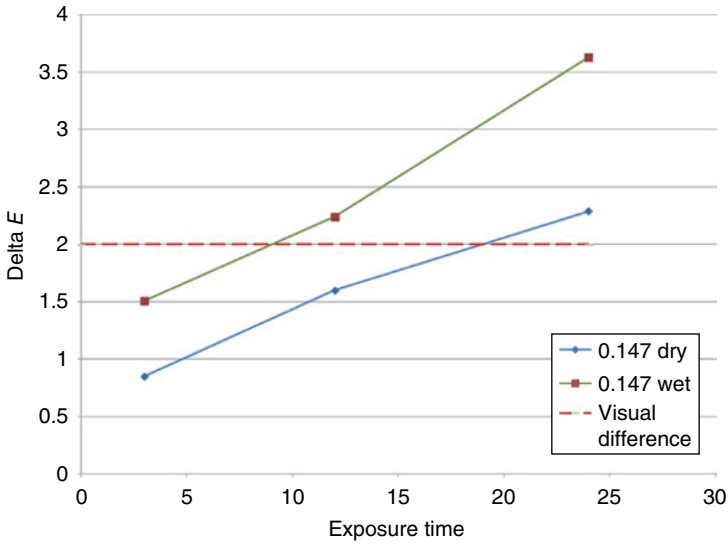


Figure 2.10 Color fade of blue capsules in light—effect of humidity. Capsules contained 0.147% indigo carmine. Delta *E* represents the total color change from initial as determined by tristimulus colorimetry.

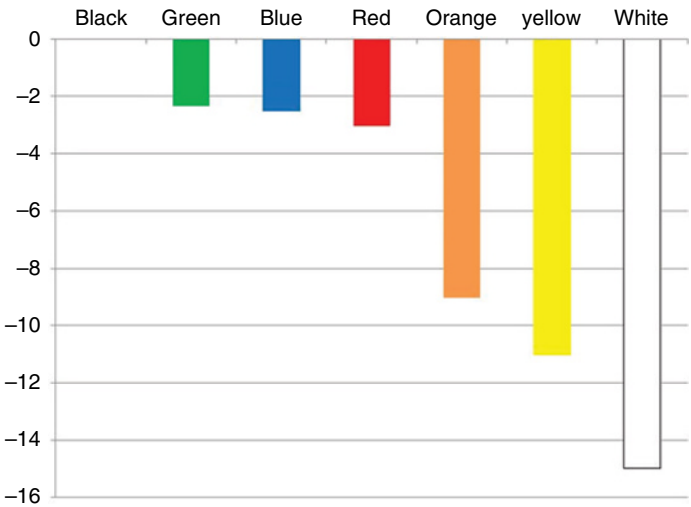


Figure 2.11 Effect of film coat color on tablet temperature. Typical temperature difference (°C) between a black sample and colored samples (after Boxhamer).

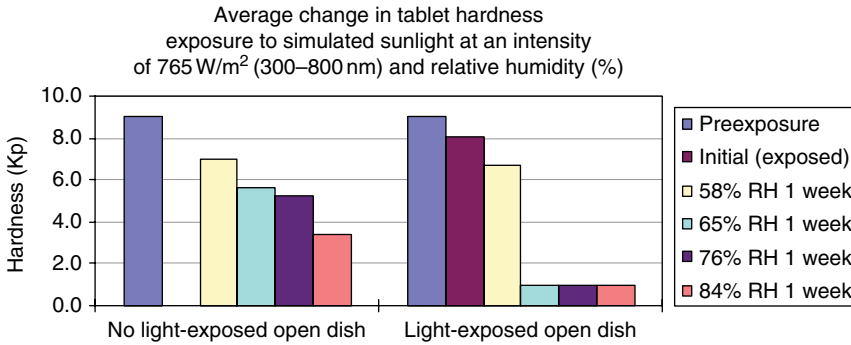


Figure 2.12 Effect of photo exposure on tablet hardness.

products to be confused. Other appearance changes include changes to glossiness of plastic components and film coats.

- Mechanical changes—Photo exposure can lead to brittleness in delivery devices, plastic components, packs, and possibly capsule shells. As shown in Figure 2.12, it can also lead to changes in tablet hardness.
- Pack changes—Light can induce changes in appearance, function, and barrier properties of packs with knock-on effects for the product stored in it.
- Changes in companion components—Dextrose infusion fluid can be degraded by light leading to the formation of reactive species that could then cause chemical instability in compounds reconstituted with it [50].

2.4.6 What About "In-Use" Conditions?

The ICH conditions are useful in providing *a* set of defined illumination conditions when considering the photostability of an API or drug product. From the industrial point of view, the Q1B requirements can be fulfilled by packing a sensitive product in a light-resistant container and labeling "protect from light." However once in the hands of a patient, the drug product may well be removed from the protective packaging. Even in a well-regulated pharmacy, there are many light sources that a product could be stored near to and in some environments where products could be deliberately removed from protective packs for display purposes. Lighting within a ward environment may consist of window-filtered daylight and/or fluorescent light, and the product may experience any mix of these depending on the physical proximity of the patient bed and medication to one of these light sources. In the case of parenteral products, the surface-to-volume ratio of the solution in the transfer line may make it particularly vulnerable to degradation.

Another factor to consider is that patients may deliberately expose themselves to sunlight either for perceived (or actual) physiological benefit or

simply because it improves mood. Finally for some formulation types, particularly topical formulations and some parenterals, there is a possibility that individualized treatments will be produced by mixing several products or diluting with diluents other than that evaluated by the industrial formulator. This could lead, for example, to dilution of a photostabilizing ingredient to below an effective level. Good practice requires the formulator to consider how the product could be used. A series of recent papers [19, 20, 58] provide guidance for in-use testing of parenteral products.

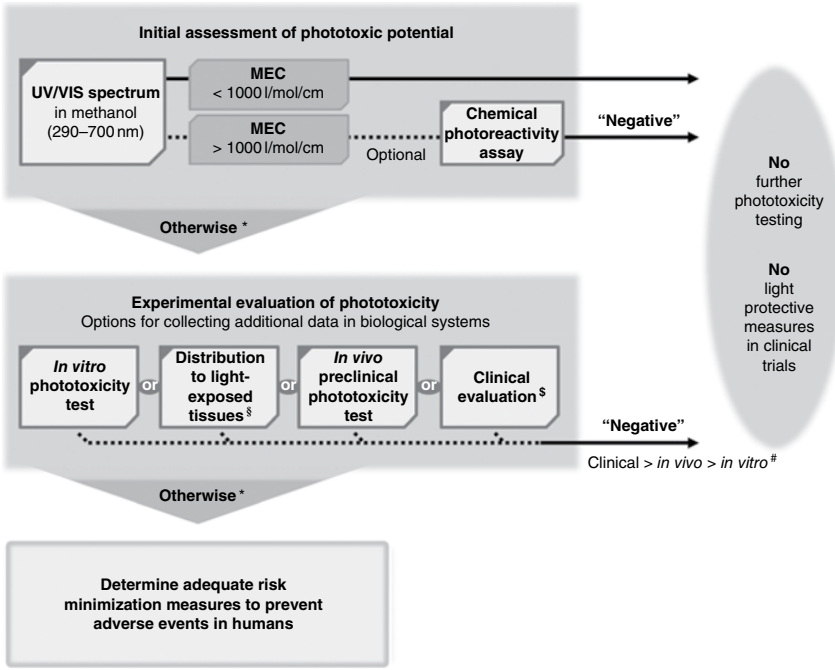


Figure 2.13 Assessment of the need for phototoxicity testing. * "Otherwise": data do not support low potential for phototoxicity or have not been generated (assay/test/evaluation not conducted). # A "negative" result in an appropriately conducted *in vivo* phototoxicity study supersedes a positive *in vitro* result. A robust clinical phototoxicity assessment indicating no concern supersedes any positive nonclinical results. A positive result in an *in vitro* phototoxicity test could also, on a case-by-case basis, be negated by tissue distribution data (see text). In the United States, for products applied dermally, a dedicated clinical trial for phototoxicity on the to-be-marketed formulation can be warranted in support of product approval. $ Clinical evaluation could range from standard reporting of adverse events in clinical studies to dedicated clinical photosafety trial. § Tissue distribution is not a consideration for the phototoxicity of dermal product.

2.4.7 Photostability Is Not All There Is to It

Photostability testing also needs to consider phototoxicity (ICH S10) [24]. There is a growing literature that shows a correlation between photostability and phototoxicity [51, 52]. In particular if the photodegradation pathway includes the generation of reactive oxygen species, that is, ROS (e.g., superoxide, singlet oxygen), this is a flag for the need for phototoxicity testing [53–57] (Figure 2.13). The ICH S10 [24] guidance proposes a ROS assay that involves irradiating a sample and assessing the effect on two indicator systems. The illumination conditions are considerably less intense than those in the ICH Q1B confirmatory test. As a guide, exposure to 250 W/m^2 in a Suntest CPS+ cabinet for approximately 35 min will provide the photo exposure required for this test. It is worth considering that by taking intermediate time points in a photostability experiment, it may be possible to gain information about the photostability, the kinetics of the reaction, and the photo-safety of the molecule in a single experiment.

References

1 ICH, Q1B, Stability Testing: Photostability Testing of New Drug Substances and Products. April 5, 2013. http://www.fda.gov/RegulatoryInformation/Guidances/ (accessed April 11, 2017).
2 ICH, Q1A (R2), Q1A(R2) Stability Testing of New Drug Substances and Products. November 2003 http://www.fda.gov/RegulatoryInformation/Guidances/ (accessed April 11, 2017).
3 Piechocki JT, Thoma K. Editors. *Pharmaceutical Photostability and Stabilization Technology*. Hoboken: CRC Press, 2007.
4 Tonnesen HH. Editor. *Photostability of Drugs and Drug Formulations*, 2nd Edition. Hoboken: CRC Press, 2004.
5 Thatcher SR, Mansfield RK, Miller RB, Davis CW, Baertschi SW. 2001. Pharmaceutical photostability: a technical guide and practical interpretation of the ICH Guideline and its application to pharmaceutical stability. *Pharm Technol Part II* 25: 50–62.
6 Aman W, Thoma K. 2003. ICH guidelines for photostability testing. Aspects and directions of use. *Pharmazie* 58: 877–880.
7 Sequeira F, Vozone C. 2000. Photostability studies of drug substances and products. Practical implications of the ICH guideline. *Pharm Technol* 24: 30–35.
8 Temussi F, Cermola F, Della Greca M, et al. 2011. A determination of photostability and photo-degradation products of indomethacin in aqueous media. *J Pharm Biomed Anal* 56: 678–683.
9 Allen NS. 1994. Photo-fading and light stability of dyed and pigmented polymers. *Polym Degrad Stab* 44: 357–374.

10 Machiste EO, Segale L, Conti S, et al. 2005. Effect of UV light exposure on hydrophilic polymers used as drug release modulators in solid dosage forms. *J Drug Deliv Sci Technol* 15: 151–157.
11 Schoolenberg GE. 1988. A fracture mechanics approach to the effects of UV-degradation on polypropylene. *J Mater Sci* 23:1580–1590.
12 Bowen L, Mangan M, Haywood A, Glass B. 2007. Stability of frusemide tablets repackaged in dose administration aids. *J Pharm Pract Res* 37:178–181.
13 Anderson NH, Byard SJ. Photostability testing: Design and interpretation of tests on new drug substances and dosage forms. In Tønnesen HH. Editor. *Photostability of Drugs and Drug Formulations*. Boca Raton: CRC Press, 1996, pp. 137–172.
14 Templeton AC, Xu H, Placek J, Reed, RA. 2005. Implications of photostability on the manufacturing, packaging, storage, and testing of formulated pharmaceutical products. *Pharm Technol* 29(3): 68–86.
15 Kristensen, S. Photostability of parenteral products. In Tønnesen HH. Editor. *Photostability of Drugs and Drug Formulations*. Boca Raton: CRC Press, 1996, pp. 303–327.
16 Tønnesen HH, Moore DE. 1993. Photochemical degradation of components in drug formulations. *Pharm Technol* 5:27–34.
17 Baertschi SW, Alsante KM, Tønnesen HH. 2010. Commentary: a critical assessment of the ICH guideline on photostability testing of new drug substances and products (Q1B). *J Pharm Sci* 99: 2934–2940.
18 Templeton AC, Klein LJ. 2007. Practical considerations for effectively conducting pharmaceutical photostability testing. *Am Pharm Rev* 10: 124–128.
19 Baertschi SW, Clapham D, Foti C, et al. 2013. Implications of in-use photostability: proposed guidance for photostability testing and labeling to support the administration of photosensitive pharmaceutical products, Part 1: drug products administered by injection. *J Pharm Sci* 102(11): 3888–3899.
20 Baertschi SW, Clapham D, Foti C, et al. 2015. Implications of in-use photostability: proposed guidance for photostability testing and labeling to support the administration of photosensitive pharmaceutical products, Part 2: topical drug products. *J Pharm Sci* 104(9): 2688–2701.
21 Blessy M, Patel RD, Prajapati PN, Agrawal YK. 2014. Development of forced degradation and stability indicating studies of drugs—A review. *J Pharm Anal* 4(3): 159–165.
22 Clapham D, Templeton AC, Klein LJ, Kleinman MH. Practical aspects of conducting photostability stress testing. In Baertschi SW, Alsante KM, Reed RA. Editors. *Pharmaceutical Stress Testing Predicting Drug Degradation*, 2nd Edition. London; New York: Informa Healthcare, 2011, pp. 218–232.
23 Smith KC. 2014. Basic Photochemistry. http://photobiology.info/Photochem.html (accessed April 11, 2017).
24 ICH, S10, Guidance on photo-safety evaluation of pharmaceuticals, December 2012. http://www.ema.europa.eu/docs/en_GB/document_library/

Regulatory_and_procedural_guideline/2012/12/WC500136404.pdf (accessed April 11, 2017).
25 ICH, Q3A (R2), Impurities in new drug substances, October 25, 2006. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3A_R2/Step4/Q3A_R2__Guideline.pdf (accessed April 11, 2017).
26 ICH, Q3B (R2), Note for guidance on impurities in new drug substances, June 2006. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002676.pdf (accessed April 11, 2017).
27 Noboru O, Robertson AR. 2006. Chapter 3. CIE Standard Colorimetric System. "3.9: Standard and Supplementary Illuminants". *Colorimetry: Fundamentals and Applications*. John Wiley & Sons, Ltd. pp. 92–96. 10.1002/0470094745.ch3.
28 Zielnik A. 2013. Photostability testing: shedding light on a not well understood guideline. *Clin Res* 5(2): 50–56.
29 Filho CA, Gomes DF, Guedes JPM, Batista RM, Santos BS. 2011. Considerations on the quinine actinometry calibration method used in photostability testing of pharmaceuticals. *J Pharm Biomed Anal* 54(4): 886–888.
30 Baertschi SW. 1997. Commentary on the quinine actinometry system described in the ICH draft guideline on photostability testing of new drug substances and products. *Drug Stand* 1: 193–195.
31 Allen JM, Allen SK, Baertschi SW. 2000. 2-Nitrobenzaldehyde: a convenient UV-A and UV-B chemical actinometer for drug photostability testing. *J Pharm Biomed Anal* 24: 167–178.
32 www.intl-lighttech.com/services/light-measurement-handbook (accessed April 11, 2017).
33 Grooff D, Francis F, De Villiers MM, Ferg E. 2013. Photostability of crystalline versus amorphous nifedipine and nimodipine. *J Pharm Sci* 102(6): 1883–1894.
34 http://www.cosmoscience.org/blog/wp-content/uploads/2014/08/GSluggett-Application-of-Topochemical-Principles-and-Solid-State-Photoreactivity.pdf (accessed April 11, 2017).
35 Tonneson H. *Photostability of Drugs and Drug Formulations*. Boca Raton: Taylor and Francis, 2006, p. 359.
36 Akimoto K, Inoue K, Sugimoto I. 1985. Photo-stability of several crystal forms of cianidanol. *Chem Pharm Bull* 33:(9) 4050–4053.
37 Albini A, Fasani E. Rationalizing the photochemistry of drugs. In: Toennesen HH. Editor. *Photostability of Drugs and Drug Formulations*, 2nd Edition. Boca Raton: CRC Press, 2004, pp. 67–110.
38 Olovsson G, Scheffer JR, Trotter J, Wu CH. 1997. Novel differences between the solid state and solution phase photochemistry of 1,2-cyclodecanedione. *Tetrahedron Lett* 38: 6549–6552.
39 Thatcher SR, Mansfield RK, Miller RB, Davis CW, Baertschi SW. 2001. Pharmaceutical photostability: a technical guide and practical interpretation

of the ICH guideline and its application to pharmaceutical stability—Part I. *Pharm Technol North Am* 25(3): 98–110.

40 Baertschi SW. 2004. Pharmaceutical Photostability Testing: Sample Presentation. Pharmaceutical Photostability 2004. PPS 04, London, UK.

41 Janssen P, Baertschi S. 2004 Studies on the Light-Catalyzed Discoloration of Tapazole Tablets, presented at Photostability 04, London, UK.

42 Atsushi U, Satomi O, Ohtake H, et al. 2015. Photochemical mechanism of Riboflavin- induced degradation of Famotidine and a suggested pharmaceutical strategy for improving photostability. *J Pharm Sci.* 10.1002/jps.24689.

43 Lu Y, Bowen WE, Reed RA, et al. Unexpected photochemistry in pharmaceutical products. In Piechocki JT, Thoma K. Editors. *Pharmaceutical Photostability and Stabilization Technology*. New York: Informa Healthcare, 2007, p. 235.

44 Wardman PL, Candeias LP. 1996. Fenton chemistry: an introduction. *Radiat Res* 145(5): 523–531.

45 Thoma K, Kubler N. New results in the photo-instability of antimycotics in drugs. In Albini A, Fasani E. Editors. *Photochemistry and Photo-Stability*. Cambridge, UK: Royal Society of Chemistry, 1998, pp. 116–133.

46 Li S, Schoenich C, Brochardt RT. 1995. Chemical instability of protein pharmaceuticals: mechanisms of oxidation and strategies for stabilization. *Biotechnol Bioeng* 48(5): 490–500.

47 Thoma K, Klimek R. 1991. Photo stabilization of drugs in dosage forms without protection from packaging materials. *Int J Pharm* 67(2): 169–175.

48 <1061> Color- Instrumental Measurement USP38 August 2015.

49 Kaur H, Mariappan TT, Singh S. 2003. Behavior of uptake of moisture by drugs and excipients under accelerated conditions of Temperature and humidity in the absence and presence of light. *Pharm Technol* 27: 52–56.

50 Brustugun J, Kristensen S, Tønnesen HH. 2005. Photosensitizing effect of 5-hydroxymethyl-2-furaldehyde in glucose infusion solution. *Pharm Europa* 17: 460–461.

51 Ferguson J, DeLeo VA. 2007. Drug and chemical photosensitivity: exogenous. *Basic Clin Dermatol* 38: 199–217.

52 Cosa G. 2004. Photo-degradation and photosensitization in pharmaceutical products: assessing drug phototoxicity. *Pure Appl Chem* 76: 263–275.

53 Kleinman MH. 2013. Using photo-reactivity studies to provide insight into the photo-safety of pharmaceutical therapies. *TrAC* 49: 100–107.

54 Kleinman MH, Smith MD, Kurali E, et al. 2010. An evaluation of chemical photo-reactivity and the relationship to phototoxicity. *Regul Toxicol Pharmacol* 58: 224–232.

55 Henry B, Foti C, Alsante K. 2009. Can light absorption and photostability data be used to assess the photo-safety risks in patients for a new drug molecule?. *J Photochem Photobiol B Biol* 96: 57–62.

56 Onoue S, Hoso K, Wakuri S, et al. 2013. Establishment and Intra-/Inter-laboratory validation of a standard protocol of reactive oxygen species assay for chemical photostability evaluation. *J Appl Toxicol* 33:1241–1250.

57 Onoue S, Hosoi K, Tsuguto T, et al. 2014. Intra-/inter-laboratory validation study on reactive oxygen species assay for chemical photo-safety evaluation using two different solar simulators. *Toxicol in Vitro* 28: 515–523.

58 Allain L, Baertschi SW, Clapham D, et al. 2016. Implications of in-use photostability testing: Proposed guidance for photostability testing and labeling to support administration of photosensitive pharmaceutical products, Part 3: Oral drug products. *J Pharm Sci* 105(5): 1586–1594.

3

ICH Q1D

Bracketing and Matrixing Designs for Stability Testing of New Drug Substances and Products

Raymond Peter Munden

Munden Consultancy, Royston, UK

3.1 Summary

Stability testing is a vital component in ensuring the ongoing quality of a pharmaceutical product once marketed and handed over to patients. It is, however, both resource and time intensive. It is therefore important to ensure that stability protocol designs are highly efficient. The ICH Q1D guidelines on bracketing and matrixing (B&M) offer ways in which this objective may be practically achieved. While, however, bracketing has long now been accepted, despite the existence of these very clear guidelines there still seems to be considerable reluctance to apply matrixed designs. This may be due to a perception both that designing matrixed studies is difficult and also that regulatory bodies will not accept such reduced stability designs.

This chapter will demonstrate how these assumptions are, in reality, incorrect. It will show how B&M can reduce the resource required while ensuring that there is no significant loss of knowledge of, and confidence in, the quality of the product. Real examples of reduced designs that have been submitted in marketing applications and accepted by assessors will be presented. The potential effects of reduced testing on shelf-life estimates will also be explored.

It should be noted that these reduced designs reduce not only the costs of testing stability samples but also the costs of sample management as fewer samples need to be stored, thereby leading to savings in the resource of labeling samples, placing them on storage and in storage costs. The latter may also mean that stability chambers do not run out of capacity and hence the high cost of purchasing new facilities may be avoided.

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3.2 Introduction

Stability testing is a vital part of product development. It is, however, quite resource consuming and time consuming. Generally a minimum of 12-month stability data is required for a new product license, and thus it is also often on the critical path of development. It is therefore vital that stability protocols are efficiently designed in order to minimize the resource required and to ensure regulatory approval.

Fortunately there are well-accepted procedures, namely, B&M, for reducing the amount of stability testing required. These are procedures for reducing the number of samples of product tested for stability that, when correctly applied, should result in neither loss of quality of the data produced nor a significant change in the predicted shelf life. Such procedures are also theoretically applicable to drug substance, but, since there tend to be fewer presentations or batches tested than for product, the opportunities and benefits are far more restricted.

Stability samples must be manufactured, labeled, and stored; therefore reducing the number of stability samples can also potentially save in sample production and management costs, especially since storage facilities are quite expensive to set up and maintain.

Bracketing, in particular, and matrixing have already been routinely applied to stability studies for some time now and indeed are the subject of an ICH paper, Q1D [1], issued in 2002. Preceding that were guidelines from the Committee for Proprietary Medicinal Products (CPMP, now CHMP), issued in 1997, giving guidance on bracketing but which were withdrawn when ICH Q1D was issued. In fact matrixing was described in papers authored by staff working at the FDA [2, 3] and the Danish Medicines Agency [4] sometime before the ICH guidelines were issued. Despite this there is still an underlying perception in industry that the regulatory authorities will be reluctant to accept reduced stability protocols. In practice the author has had experience of submitting many reduced stability protocols, with both bracketing and matrixing applied, without encountering any resistance from the assessors; indeed, some were praised for their good design. The author has also had conversations with regulators who have queried why more submissions do not include reduced stability designs, particularly matrixing.

3.3 Definitions

Bracketing and matrixing are carried out across different presentations of an active in a given product type, that is, across different factors such as strength, pack, fill volume, packing site, etc. The definitions as given in ICH are as follows.

3.3.1 Bracketing

"The design of a stability schedule such that only samples on the extremes of certain design factors, for example, strength, package size, are tested at all time points as in a full design. The design assumes that the stability of any intermediate levels is represented by the stability of the extremes tested."

This means that, as an example, for a product with three different strengths, say, tablets at 2, 4, and 6 mg, it may be possible to omit testing of the 4 mg tablets.

3.3.2 Matrixing

"The design of a stability schedule such that a selected subset of the total number of possible samples for all factor combinations is tested at a specified time point. At a subsequent time point, another subset of samples for all factor combinations is tested. The design assumes that the stability of each subset of samples tested represents the stability of all samples at a given time point."

This means that at a given time point (other than the initial or final time points), every batch on stability need not be tested.

3.4 Applicability

B&M can be applied to virtually any formulation, although for more complex delivery systems with a large number of potential drug–device interactions, such as metered-dose inhalers (MDIs), MDPIs, and transdermal delivery systems, additional justification is very likely to be required. Indeed, the FDA is unlikely to accept reduced designs for inhaled devices although other regulatory bodies have accepted them.

The Q1D guidelines give some guidance on the applicability of B&M. The existence of multiple strengths of a formulation is an obvious target for such reduced designs, and the guidelines specifically allow this without justification where the different strengths have identical formulations, for example, tablets with identical formulations but different compression weights or capsules of different strengths made with identical power blends but different fill plug sizes. Different strengths made with closely related formulations can be subject to B&M with some justification. What constitutes a closely related formulation is not defined, but examples could be the following:

Different strengths are obtained by minor changes in one excipients (say, for 2, 4, and 6 mg tablets where the amount of one excipient changes to accommodate the varying active).
Different coatings on otherwise identical tablets.

The justification might be a statement confirming either that it has been experimentally demonstrated that varying the excipient or coating has no effect upon the active or that earlier development stability studies showed the changes unlikely to have any effect upon stability.

B&M can also be applied to studies of the same closure system where either container size or fill varies. When applying bracketing, care needs to be taken to ensure that the intermediate condition is correctly identified. This is because it might not necessarily be that the largest and smallest containers represent the extreme configurations. Consideration should also be given to wall thickness, closure geometry, surface area to volume ratio, headspace to fill volume ratio, water vapor, and oxygen permeation rate per dosage unit.

Even when the closure varies, B&M is possible with some justification. Such justification might be demonstration that the product is not sensitive to water or a discussion of the relative permeation rates of the closure systems.

Many regulatory authorities welcome the opportunity to comment on stability protocols prior to formal submissions, and it may be wise to take advantage of this option, particularly if the design isn't entirely straightforward.

B&M can still be applied when all batches cannot be placed on storage simultaneously. The allowable difference in start times should be decided on a case-by-case basis as a business risk, since identification of any stability differences between batches will be delayed. As a suggestion, up to 2 months, maximum spread appears reasonable. Clearly, if studies are not started simultaneously, the efficiency gains will be significantly reduced.

3.5 Designs

3.5.1 Bracketing

Bracketing designs are fairly straightforward and have been around for longer than matrixed designs. A typical example of bracketing, as was given in the withdrawn CPMP guideline previously mentioned, is reproduced as Table 3.1. This example is a product available in three different strengths, two different packs, and, for one of these packs, three different sizes. In this instance it would be necessary to demonstrate that the 30 and 500 HDPE sizes truly bracket the 100 size as discussed earlier.

If subsequent to commencing studies it is decided not to register one of the extreme presentations, it is permissible to maintain the stability study in order to support any intermediate presentations that have not been placed on stability. A commitment should then be provided to place the omitted presentations on stability.

Table 3.1 Example of a bracketing design.

	Dosage strength and batch of active (A, B, C)								
	50 mg			75 mg			100 mg		
Pack type	A	B	C	A	B	C	A	B	C
Blister	X	X	X	O	O	O	X	X	X
HDPE/30	X	X	X	O	O	O	X	X	X
HDPE/100	O	O	O	O	O	O	O	O	O
HDPE/500	X	X	X	O	O	O	X	X	X

A, B, C, batches; O, not tested; X, batch tested.

3.5.2 Matrixing

For matrixing each storage condition should be treated separately under its own matrixing design. Accelerated conditions can therefore theoretically be matrixed, but as only three time points including initial and final must be analyzed, gains are minimal and it may well be considered preferable to carry out full testing since accelerated conditions may provide early warning of any unexpected events.

As far as possible a matrixing design should be balanced so that each presentation (of strength, pack, fill volume, etc.) is tested to the same extent over the intended study period. It is a requirement, however, that full testing must be carried out at the last time point at the time of submission, frequently rendering exact balancing impossible. The design should, however, be at least approximately balanced.

Examples of simple matrixed designs for a product in two strengths are shown in Table 3.2 (assuming submission with 12-month data). Table 3.2a shows a one-half matrix design (or one-half reduction) and Table 3.2b a two-third design (one-third reduction). Since full testing is required at beginning and end of the stability program, and also at the last time point at the time of submission, the actual reductions in the number of test samples are smaller, being 31 and 21%, respectively. These examples are for definitive stability and hence three batches are tested. Fewer batches may be permissible for follow-up stability or for new products of an existing active pharmaceutical ingredient (API), but matrixing is still allowable.

Note that these designs have assumed submission with 12 months data and 36 months as the longest time point. The 36 month (and 48 month) time point could also be matrixed if intending to test up to 48 months (or 60 months).

Note that in Table 3.2b interchanging the Xs and Os, excluding the initial, 12 month and final time points, makes it a one-third matrix (two-third

Table 3.2 Matrix designs for two strengths.

Time presentation		0	3	6	9	12	18	24	36
(a) One-half matrix									
S1	Batch 1	X	X	O	O	X	O	X	X
	Batch 2	X	O	X	X	X	O	X	X
	Batch 3	X	O	X	O	X	X	O	X
S2	Batch 1	X	O	X	X	X	X	O	X
	Batch 2	X	X	O	O	X	X	O	X
	Batch 3	X	X	O	X	X	O	X	X
(b) Two-third matrix (one-third reduction)									
S1	Batch 1	X	O	X	X	X	X	X	X
	Batch 2	X	X	O	X	X	O	X	X
	Batch 3	X	X	X	O	X	X	O	X
S2	Batch 1	X	X	O	X	X	O	X	X
	Batch 2	X	X	X	O	X	X	O	X
	Batch 3	X	O	X	X	X	X	X	X

O, not tested; X, tested.

reduction, a 42% reduction in practice). The reader therefore now has matrix designs to apply to most examples that they will probably encounter.

With matrixing, designs can be complete (matrixing on time points alone), meaning that all batches are tested in all possible presentations, or incomplete (matrixing on time points and factors), whereby some batches are not tested in some presentations. The larger the number of different presentations, the more likely it is that an incomplete design is acceptable. An example of a more complex design, with 6 factors (3 strengths and 3 packs) using a one-half design with full testing at 12 months, is given in Table 3.3. Table 3.3a shows a complete reduced design and 3.3b an incomplete reduced design. In Table 3.3b, while all combinations of strength and pack are tested, each individual batch of product is not tested in all strength/pack combinations.

ICH Q1D gives further example designs. It should be noted that it is permissible to combine both bracketing and matrixing in a single design.

If during the course of a matrixed stability study it becomes apparent that the matrix is no longer appropriate, for example, if the required expiry period (EP) might not be achieved, you can revert to full testing or to a smaller reduction in testing. Once, however, matrixing is reduced, it is not permissible subsequently

Table 3.3 Matrix design for 3 strengths and 3 packs.

	1			2			3		
Strength pack	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
(a) Complete reduced design (matrixing on time points alone)									
Batch 1	T1	T2	T3	T1	T2	T3	T1	T2	T3
Batch 2	T2	T3	T1	T2	T3	T1	T2	T3	T1
Batch 3	T3	T1	T2	T3	T1	T2	T3	T1	T2
(b) Incomplete reduced design (matrixing on time points and factors)									
Batch 1	O	T2	T3	T1	T2	T3	T1	T2	T3
Batch 2	T2	T3	T1	T2	O	T1	T2	T3	T1
Batch 3	T3	T1	T2	T3	T1	T2	T3	T1	O

Key								
	0	3	6	9	12	18	24	36
T1	X	X	O	O	X	O	X	X
T2	X	O	X	X	X	O	X	X
T3	X	O	X	O	X	X	O	X

O, not tested; X, tested.

to reinstate the original matrix. If there is any doubt about the likely stability of a product, it might therefore be good practice to put sufficient samples on stability for a full program. In practice for full stability, extra samples are usually stored to allow for any repeat analyses, so when storing samples for a reduced design you could consider storing only sufficient samples for full testing, or just a very small excess, to save on samples stored yet still have enough to cover full testing should it be required.

It is also worth noting that should the worst happen, such that when reaching a key storage time it is found that the reduced data does not support the required shelf life; then extra replicate analyses can be carried out in order to obtain an improved shelf-life estimate.

Different tests may, in principle, be matrixed to different extents. For example, if the test for active content is highly reproducible and the active is stable, then this test may be subjected to a one-half, or possibly even a one-third, matrix design. If, however, the test for a key impurity shows significant variability and there is an indication that the impurity might increase on storage, then a two-third matrix, or possible no matrix at all, may be more appropriate for this test.

For simplicity, however, a practitioner may find it preferable to matrix all tests to the same design as for that test demanding the minimum reduction.

3.5.3 Degree of Reduction

The degree of reduction in testing is dependent on the variability between, and homogeneity within, batches and the precision of the test method, with low variability supporting greater matrixing reductions. Also, generally, greater numbers of presentations support larger test reductions. The inherent stability of the formulation is also very important as reductions in the number of samples tested may result in wider confidence intervals and as a result a shorter shelf-life estimate. Therefore if a product is likely to have an inherently short shelf life, then reductions in testing should be minimal, while greater reductions may be appropriate for a product likely to significantly exceed a 36-month shelf life.

The Accelerated Stability Assessment Program [5], as described in Chapter 4 of this book, could be an excellent aid to predicting stability in order to justify matrixing and bracketing. A risk-based approach to stability studies such as this has been proposed [6].

Table 3.4 provides some qualitative feel for the reductions that may be applied according to variability and anticipated EP.

Table 3.4 Degree of reduction in bracketing and matrixing.

Stability Variability[a]	Good EP > 36 months	Moderate EP ~ 24 months	Poor EP ≤ 12 months
Very small	* * * * *	* * *	* *
Moderate	* * * * * *	* * *	*
High	* * *	*	*

[a] Variability between batches, assay, and/or test results.
EP, expiry period.

Large * * ← Size of reduction → * * Small or none

3.6 Data Evaluation

Stability data from bracketed and/or matrixed studies should be treated in the same way as data from nonreduced studies and as described in ICH Q1E [7]. If inspection of the data shows clearly that there is no significant change in a parameter, then no statistical analysis is required. Where statistical analysis is required, the data from different batches and presentations should first be tested for poolability. If pooling is not justified, then the shortest EP obtained should be applied to all batches and presentations. If pooling is justified, then the predicted EP applies to all presentations. Note that pooling may be rejected if a small statistically significant difference in slopes is detected but that may be of no practical significance. Liu and coworkers [8] offered an alternative approach to evaluating poolability to allow for this.

3.7 Practical Examples

Some examples of reduced stability designs that were accepted by regulatory authorities are now given. In each case these particular designs were accepted without significant comment. The testing regimes provided represent the long-term stability testing conditions chosen for the products. The accelerated testing conditions, as per comment in Section 3.5.2, were not matrixed. Products are not identified in order to protect confidentiality.

3.7.1 Conventional Tablet Product, New API

Example 1 shown in Table 3.5 is for a simple immediate release tablet of strength 1 mg for new submission to the FDA. Stability data was available on higher strength tablets, with very similar formulations, which had showed very good stability but weren't to be marketed. Two packs were to be used, double foil blister and HDPE bottles. The bottles were in two sizes, 50 and 80 cc, and each had low and high fill volumes, although it was thought likely that only one bottle size would actually be marketed. Batches 1, 2, and 3 included different input batches of API. The design submitted applied one-half matrices and a standard design using three batches for the blister pack. It is worth highlighting that for the HDPE bottle only two batches for each different presentation were placed on stability, whereas for a new API three are required according to the ICH guidelines. It is certainly arguable that, as the presentations are so similar, in practice three different batches are being used. In fact not only was this design accepted by the FDA, but also the reviewer actually praised it as a very good design.

Another notable aspect of the design to note is the distribution of batches for the bottle pack. While in this design it was impossible to use each batch at the same number of times across the four different presentations, the balance is as

Table 3.5 Example 1: A matrixed design for conventional tablets 1 mg.

		Storage time (months)									
Tablet count	**Batch number**	**Initial**	**3**	**6**	**9**	**12**	**18**	**24**	**36**	**48**	**60**
(a) 50 cc bottle pack											
Low	1	X			X	X			X		(X)
Low	2	X	X	X		X	X	X	X	(X)	(X)
High	1	X		X		X		X	X		(X)
High	3	X	X		X	X	X		X	(X)	(X)
(b) 80 cc bottle pack											
Low	1	X	X	X		X	X	X	X		(X)
Low	3	X			X	X			X	(X)	(X)
High	2	X	X		X	X	X		X	(X)	(X)
High	3	X		X		X		X	X		(X)
(c) Blister pack											
	1	X	X			X		X	X	(X)	(X)
	2	X		X	X	X		X	X		(X)
	3	X		X		X	X		X		(X)

good as is possible, with batches 1 and 3 each being used in three studies and batch 2 in two studies.

3.7.2 Conventional Tablet Product, Existing API

This API had already been approved in both Europe and the United States for 200 mg tablets manufactured in Japan and packed in pack P1 at site S1 in the United Kingdom. It was now required to add 400 and 600 mg tablets manufactured in the United Kingdom and packed at two new sites, P2 and P3, with site P1 being dropped. The 200 mg tablets had shown very good stability. The stability protocol design submitted is shown in Table 3.6 and applies a one-half matrix design. It is worth noting that as this was an existing API, only two batches of each presentation were placed on testing and the submission was made with 6-month data, which was accepted by the FDA.[1]

1 This design was submitted long before the FDA guidelines for stability testing for ANDAs [9] were issued and which indicated that for existing APIs and products, as with new APIs, three batches are to be used for stability. At the time of writing it was not clear, however, whether if there are several presentations of effectively the same formulation, then using two batches of each presentation may be permissible, an argument that the author considers to be valid and justifiable since more than two batches will be studied in total.

Also noteworthy is that 200 mg tablets manufactured at site S1 were not tested on the basis that stability had already been submitted. However, those 200 mg tablets had only been studied in pack P1 and not P2 and P3, so in hindsight a better design might have included those presentations and a bracketed design adopted with the 400 mg tablets eliminated from the testing while maintaining the matrixing.

3.7.3 Metered-Dose Powder Inhaler, Existing API

This product was registered and marketed in many countries and was to be introduced to Canada, which required specific batches. The product was to be marketed in four different doses (D1 to D4 with D1 and D4 being the highest and lowest doses, respectively). The stability design was as shown in Table 3.7 and is unusual in that it uses two batches each of the highest and lowest strengths and one batch each of the two intermediate batches, a design that the designer

Table 3.6 Example 2: A matrixed design for conventional tablets 200, 400, and 600 mg.

	200 mg		400 mg			600 mg				
Batch	**S2,P2**	**S2,P3**	**S1,P2**	**S2,P2**	**S1,P3**	**S2,P3**	**S1,P2**	**S2,P2**	**S1,P3**	**S2,P3**
1	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
2	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1
	0	3	6	9	12[a]	18[a]	24[a]	36	48	60
T1	X		X		X		X		X	X
T2		X	X	X		X		X		X

[a] Full testing will be applied at the longest time point at the time of submission.

Table 3.7 Example 3: A design for metered-dose inhaler with four strengths.

		Storage time (months)						
Strength	**Batch**	**0**	**3**	**6**	**9**	**12**	**18**	**24**
D1	1	X	X		X		X	X
D1	2	X		X		X	X	X
D2	1	X	X		X		X	X
D3	1	X		X		X	X	X
D4	1	X	X		X		X	X
D4	2	X		X		X	X	X

termed as partial bracketing. For a new submission, with three batches being required, a full bracketing design with three batches each at the highest and lowest strengths would have been more conventional, but the design in Table 3.7 provides real data on all presentations for the same number of batches.

The design also applies a one-half matrix. Because the submission was not required until the 18-month data were available and a 24-month maximum shelf life was required, full testing is programmed in at the 18- and 24-month time points.

Testing of inhaled devices requires significantly more resource than for most other formulation types, so, in this design, reducing the number of batches tested from potentially 12 (3 batches of 4 presentations) to 6, and the further reductions by matrixing represent savings that could be measured in £100,000s. It is therefore very unfortunate that, as mentioned in Section 3.4, the FDA is very unlikely to accept reduced designs for metered-dose inhaled products.

3.7.4 Conventional Tablet Product, New API

These tablets were to be in a single strength in HDPE bottles with three different presentations. These were 60 cc bottles containing from 3 to 7 tablets (a sample pack), a 60 cc bottle containing 30 tablets, and a 100 cc bottle containing 90 tablets. The submission time was uncertain but was expected to be with either 18- or 24-month data.

The design is shown in Table 3.8 and includes both bracketing and matrixing. For the 60 cc bottles the extremes are represented by three tablets in 60 cc and 90 tablets in the 100 cc bottle; consequently the 60 cc bottles containing four to seven tablets are bracketed out. Three batches each of the remaining presentations were used and additionally a one-half matrix applied. Because the

Table 3.8 Example 2: A matrixed design for conventional tablets, new API.

	3 tablets	30 tablets	90 tablets
Batch	**60 cc bottle**	**60 cc bottle**	**100 cc bottle**
1	T1	T2	T1
2	T2	T1	T2
3	T1	T2	T1

	0	3	6	9	12	18[a]	24[a]	36
T1	X		X		X		X	X
T2	X	X		X		X		X

[a] Full testing depending upon submission time.

submission point was not certain, flexibility for full testing at the longest time point before submission was allowed for.

3.8 Effect of Matrixing Upon Shelf-Life Estimations

Reducing testing will of course reduce the data available, and so it is important to consider the effect of reducing testing upon shelf-life estimations. As confidence intervals tend to widen with reduced data, then the most likely risk is that shelf lives will be underestimated, posing a business risk rather than a patient risk. Golden et al. [10] carried out some studies to simulate the effect of matrixing. In this study they took two products (a capsule product and a MDI product) that had full results from completed 36-month stability programs, and then they retrospectively created artificial one-half reduced matrices, designed as shown in Table 3.2a for strength 1 (S1), by simply leaving out appropriate data. Shelf-life estimates were then made by the conventional statistical linear regression analysis of confidence intervals intercepting specification limits. Because in practice there had been full testing, there were six

Table 3.9 Effects of matrixing upon shelf-life estimations.

	Batch permutation							
	Shelf-life estimation (months)							
Test	**Full**	**123**	**132**	**213**	**231**	**312**	**321**	**Mean**
(a) Metered-dose inhaler product								
API content	23	24	24	21	25	29	29	25
Dissolution	>36	>36	>36	>36	>36	>36	>36	>36
Impurities	17	18	18	21	22	18	14	19
Minimum[a]	17	18	18	21	22	18	14	
(b) Capsule product								
Dose	34	29	29	29	27	>36	32	30
Sum	25	19	20	19	25	18	25	21
Impurities	>36	>36	26	>36	25	>36	>36	33
Minimum[a]	25	19	20	19	23	18	25	

Table shows the statistical shelf-life estimation in months for both full testing and for each of the six possible reduced permutations and the average of all six permutations.
Dose, delivered dose; sum, fine particle mass by cascade impaction (stages 4–6).
[a] Minimum shelf life from all tests.

possible one-half matrices that could be simulated (i.e., the batches could have been in the order 123, 213, or 231 etc.), so each possible permutation was simulated. This was done for each of three different tests with the results shown in Table 3.9. It can be seen that there is some variation in shelf-life estimate, but for reduced testing with the MDI the estimates never exceeded the estimate from full testing, as would be expected. The capsules did show some extended shelf-life estimates but it is worth noting that when this analysis was done, in 1996, there was not the requirement for full testing at the longest time point at submission; hence in this analysis the 12-month time point was also matrixed. In practice had full testing at 12 months been included in the simulation, as would now happen, that would have likely tightened the estimates further.

Since the Golden paper was published, there have been numerous similar studies, including a study of artificial data producing 1000 simulations [11], many of which were unfortunately unpublished. These studies have shown that when properly carried out, matrixing has minimal effect upon shelf-life estimations. Similar studies have also been applied to show that matrixing can be applied to combination products [12].

3.9 Conclusion

The use of B&M for optimizing the design of stability protocols has been described. This can potentially result in considerable savings in the costs of carrying out stability studies; as the amount of testing required is reduced, fewer samples have to be labeled and placed on storage, and consequently the space required to store them is also reduced.

The procedures are easy to apply, well established, and accepted by the regulatory authorities and are described in ICH guidelines. There is, therefore, no reason why their application should not be widespread throughout the pharmaceutical industry.

References

1 International Conference on Harmonisation. (2002). Bracketing and Matrixing Designs for Stability Testing of New Drug Substances and Products. *ICH Q1D.*
2 Nordbrock, E. (1992). Design and Analysis of Stability Studies. *J Biopharm Stat*, 2, 91–113.
3 Fairweather, W., Lin, T.D., Kelly, R. (1995). Regulatory, Design and Analysis Aspects of Complex Stability Studies. *J Pharm Sci*, 84, 1322–1326.
4 Helboe, P. (1992). New Design for Stability Testing Programs: Matrix or Factorial Designs. *Drug Inf J*, 26, 629–634.

5 Waterman, K.C., Colgan, S.T. (2008). A Science Based Approach to Setting Expiry Dating for Solid Drug Products. *Regul Rapporteur*, 5, 7/8.

6 Colgan, S.T., Timpano, R.J., Roberts, M., Weaver, R., Ryan, K., Fields, K.W., Scrivens, G. (2014). Opportunities for Lean Sigma Stability Strategies. *J Pharm Innov*, 9, 259–271.

7 International Conference on Harmonisation. (2003). Evaluation for Stability Data. *ICH Q1E*.

8 Liu, J., Tung, S., Pong, Y. (2006). An Alternative Approach to Evaluation of Poolability for Stability Studies. *J Biopharm Stat*, 16, 1–14.

9 U.S. Department of Health and Human Services, Food and Drug Administration Center for Drug Evaluation and Research (CDER). (2013). Stability Testing of Drug Substances and Products. *Guidance for Industry ANDAs*.

10 Golden, M.H., Cooper, D., Riebe, M., Carswell, K. (1996). A Matrixed Approach to Long-Term Stability Testing of Pharmaceutical Products. *J Pharm Sci*, 85, 240–245.

11 Pong, A., Raghavarao, D. (2000). Comparison of Bracketing and Matrixing Designs for a Two-Year Stability Study. *J Biopharm Stat*, 10(2), 217–228.

12 Pong, A., Raghavarao, D. (2002). Comparing Distributions of Shelf Lives for Drug Products with Two Components Under Different Designs. *J Biopharm Stat*, 12(3), 277–293.

4

ICH Q1E Evaluation for Stability Data

Garry Scrivens

Pfizer, Sandwich, UK

4.1 Introduction

The purpose of the ICH Q1E guideline is to provide recommendations on how stability data can be used to establish retest periods for drug substances or shelf lives for drug products. The ICH Q1E guideline is intended to be used in conjunction with its "parent" guideline, ICH Q1A(R2) [1]. The parent guideline provides guidance on considerations such as selection of batches, container closure system, testing frequency, and storage conditions, while ICH Q1E provides guidance on data evaluation such as how to leverage information from accelerated studies and provides examples of statistical approaches for stability studies comprising multiple strengths and packaging types.

ICH Q1E provides recommendations on how stability data obtained from accelerated, intermediate, and long-term stability conditions can be used to propose retest periods and shelf lives in registration applications. In particular, the guideline outlines how retest periods and shelf lives can be set based on extrapolations of long-term data depending on the nature of the accelerated or intermediate stability data available. The guideline also covers topics such as bracketed and matrixed stability study designs (together with ICH Q1D [2]) and tests for the poolability of stability data from multiple batches and how this can affect the proposed retest period or shelf life.

ICH Q1E states that an appropriate statistical method should be employed to analyze the long-term primary stability data. The purpose of this chapter is not to provide a detailed discussion on the statistical approaches recommended in ICH Q1E since excellent and detailed reviews on this topic are already available [3]. In this chapter, the underlying scientific principles of ICH Q1E (and to some extent ICH Q1A(R2)) are discussed and evaluated with the intent of

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encouraging stability data to be interpreted not merely as sets of numbers to be processed purely on a statistical basis, but also to be understood and rationalized on the basis of the product's chemical and physical characteristics. A deeper understanding of *why* the stability data are the way they are is in line with the principles of quality by design and can help to ensure that stability data from multifactor studies are pooled and bracketed appropriately. In addition, this deeper understanding can help to risk assess post-approval changes such as changing packaging and climatic zone and can help to quantitatively risk assess excursions and in-use scenarios.

Firstly, the effect of environmental factors on stability is discussed; the main objective of Section 4.2 is to highlight how a more *quantitative* understanding of the effects of temperature and humidity can help to discuss and present stability data from accelerated, intermediate, and long-term conditions in a more connected and holistic fashion. This quantitative understanding, in combination with approaches for calculating the relative humidity (RH) inside packaging, enables data from multifactor stability studies comprising different packaging types to be understood and presented in a concerted and scientific manner.

Section 4.3 focuses on multifactor stability studies. The examples of "factors" provided by ICH Q1E are strength, container size, and container fill; in this section, a discussion is provided on how each of these factors can be expected to influence stability performance.

In Section 4.4, "Data Evaluation and Statistical Considerations", a variety of topics are covered including discussions on the potential causes of nonlinear stability curves, poolability criteria, and the possible effects of rounding data on the shelf-life assignment.

ICH Q1E states that where differences in stability are observed among batches or among other factors (to which we can add environmental factors), a discussion should be provided to "address the cause for the differences and the overall significance of such differences on the product." Hopefully this chapter will provide assistance to those wishing to provide such a discussion.

Finally it is necessary to acknowledge that the majority of this chapter focuses on chemical degradation (which includes growth of degradation products and loss of assay). The reason for this is that other potential shelf-life-limiting attributes (such as dissolution) less frequently exhibit stability behavior that can be reliably trended and understood in terms of underlying quantitative models. With chemical degradation, however, such models are better established.

4.2 The Effect of Environmental Factors on Pharmaceutical Stability

The parent guideline ICH Q1A(R2) states that a purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such

as temperature, humidity, and light. The following is a brief discussion on how the different environmental factors are known to affect pharmaceutical stability; a discussion on the effect of light is not included since this is covered in ICH Q1B [4] (Chapter 2), but a brief section on oxygen as an environmental factor has been included.

4.2.1 Temperature

Temperature has a profound effect on pharmaceutical stability: it affects the rate of chemical reactions and the rates of diffusional processes (such as moisture permeability through packaging) and can change the physical state of the sample. In simple scenarios, the temperature dependence of chemical reaction rates can be expected to follow the Arrhenius equation:

$$k = A.\exp\left(\frac{-E_a}{R.T}\right) \tag{4.1}$$

where

k is the rate of reaction
A is a constant referred to as the "pre-exponential factor"
E_a is the activation energy of the reaction (typically measured in $kJ.mol^{-1}$ or $kcal.mol^{-1}$)
R is the universal gas constant ($8.314\,J.K^{-1}.mol^{-1}$ or $1.987\,cal.K^{-1}.mol^{-1}$)
T is the absolute temperature (measured in kelvin)

Similarly, moisture permeation through packaging is generally observed to follow an Arrhenius-like temperature dependence, for example:

$$P = P_0 \exp\left(\frac{-E}{R.T}\right) \tag{4.2}$$

where

P is the permeability of a material (units of mass of water per unit time per ΔRH)
P_0 is a constant dependent upon the nature of the packaging material

No explicit mention of the Arrhenius equation is made in ICH Q1E nor its parent guideline ICH Q1A(R2), but the Arrhenius equation underpins the general principles of these guidelines. For example, ICH Q1A(R2) makes reference to mean kinetic temperature (MKT), which is based on an assumption of Arrhenius temperature dependence [5], and the recommended durations of accelerated stability studies are consistent with the Arrhenius equation. However, until recently, the Arrhenius equation has not generally been

explicitly applied to stability studies and the expiry dating process—partly because regulatory guidelines make no reference to Arrhenius, partly because many of the stability-related processes are physical changes that would not generally be expected to conform to Arrhenius-like temperature dependence, and partly because even for processes that can be expected to conform (such as chemical degradation), the Arrhenius equation appeared not to have the accuracy required to be applied in a reliable and quantitative manner. Many of the reasons for this apparent lack of accuracy are associated with the difficulties in applying Arrhenius to solid-state samples, for example:

- Humidity is well known to affect the rate of degradation, but the Arrhenius equation does not include a humidity term as an input.
- The Arrhenius equation requires *k*, the reaction rate to be determined at different temperatures, but the problem is that the shapes of degradation profiles in the solid state often do not conform to simple rate equations that would allow a straightforward calculation of *k*.
- In the solid state, changing temperature sometimes brings about physical changes in the sample (e.g., a melt or a glass transition), which can result in a step change in the degradation kinetics that prevents the Arrhenius equation from being accurately extrapolated. The ICH Q1E guideline cites two examples of how accelerated conditions (i.e., elevated temperature) can bring about physical changes, which results in stability behavior that is not representative of behavior under long-term storage conditions: the melting of suppositories designed to melt at 37°C and the cross-linking of gelatin capsules that is unrepresentatively rapid under accelerated conditions because the elevated temperature results in a glass transition in the gelatin.
- In situations where there the observed degradation results from multiple consecutive or competing processes, the observed degradation is unlikely to follow an Arrhenius temperature dependence.

Despite these potential pitfalls, the application of the Arrhenius equation has, in recent years, enjoyed somewhat of a renaissance in its application to solid-state pharmaceutical materials (both drug substances and drug products); this is because methods have been developed that can partly overcome these challenges—particularly those associated with the first two bullets. A number of studies including many from our own laboratories have investigated the effect of humidity on the rate of pharmaceutical chemical degradation [6–8] and have observed that if the RH is kept constant, then the Arrhenius equation is often applicable, that is:

$$k_{\mathrm{RH}} = A_{\mathrm{RH}}.\exp\left(\frac{-E_{\mathrm{a}}}{R.T}\right) \tag{4.3}$$

where

k_{RH} is the rate of reaction at a specified RH
A_{RH} is a constant, with the "pre-exponential factor" applicable to the specified RH

This is discussed in more detail in the "Humidity" section, but this together with methods to overcome problems associated with extracting a rate constant, k from experimental data (the second bullet), which are discussed in more detail in the Section 4.4.1, has allowed the Arrhenius equation to be applied more accurately and quantitatively to hundreds of drug substances and drug products; Section 4.2.6.3 "Stability Protocol Design" provides details on how the Arrhenius equation parameters can be determined. The activation energy, E_a, can be thought of as a "temperature sensitivity parameter": the higher the activation energy, the greater the effect temperature has on the rate of the reaction. The application of the Arrhenius equation has a number of benefits, for example, it allows a better understanding of how stability data from accelerated studies relates to long-term stability performance, it enables the effect of short-term excursions outside the label storage conditions to be evaluated much more accurately and quantitatively, and it allows appropriate interim expiry periods to be set on the basis of short-term accelerated studies; these are all key objectives of ICH Q1E and its parent guideline ICH Q1A(R2).

4.2.2 Humidity

It is well established that humidity can affect the rate of degradation of solid-state pharmaceuticals; this is evidenced by the fact that reference to and control of the storage RH is engrained throughout the parent guideline ICH Q1A(R2). However, there is no universally accepted "humidity analogue" of the Arrhenius equation that quantitatively links reaction rate with humidity, but this has been the topic of many investigations over the years [5, 9, 10]. The most commonly reported relationship between humidity and degradation rate is that log(degradation rate) appears to increase linearly with RH, that is, that degradation rate increases exponentially with RH:

$$\log(\text{degradation rate}) = \text{constant} + B(\text{RH}) \tag{4.4}$$

that is,

$$\text{Degradation rate} = \text{constant} \times \exp(B.\text{RH}) \tag{4.5}$$

where B is a parameter fitted from experimental data and is a "humidity sensitivity" parameter: higher values of B reflect higher sensitivity to effects of humidity.

Other relationships between degradation rate and humidity level have been reported: degradation rate is proportional to the humidity raised to the power

of a constant [11], that is, a linear relationship between log(degradation rate) and log(*H*) is expected, where *H* is a measure of the humidity level of the storage environment (e.g., RH, absolute humidity, vapor pressure) or of the moisture level in the solid sample (e.g., water activity or water content). Despite the mathematical differences of these models, it can be difficult experimentally to discern which model best describes the effects of humidity due to experimental variability, complications arising from nonlinear degradation, and the slowness of degradation under some conditions. However, the "exponential" model (i.e., Equations 4.4 and 4.5, rather than the "power" model) currently appears to be favored by most researchers in this area.

4.2.3 The Combined Effects of Temperature and Humidity

At constant temperature, RH, absolute humidity, and water vapor pressure are linearly correlated, which means that it does not matter which of these measures of humidity are used to model the relationship between degradation rate and humidity (e.g., as modeled in Equation 4.4). However, when modeling the combined effects of temperature and humidity on the rate of degradation, different humidity descriptors result in different models.

Genton et al. [6] and Waterman et al. [7–9] proposed a combined temperature and humidity model in which *RH* is used as the humidity descriptor:

$$\text{Degradation rate} = A \times \exp\left(\frac{-E_a}{R.T}\right) \times \exp\left(B.\text{RH}\right) \tag{4.6}$$

that is,

$$\log\left(\text{degradation rate}\right) = \log A - \frac{E_a}{R.T} + B.\text{RH} \tag{4.7}$$

This model incorporates an Arrhenius dependence on temperature together with the effects of humidity described previously (Equations 4.4 and 4.5). There has been no detailed theoretical argument put forward to rigorously explain this model—the model is largely empirical in nature, but it has been argued that RH affects molecular mobility and hence increases the collision frequency and the pre-exponential constant of the Arrhenius equation [5]. Equation 4.7 may be visualized in terms of a three-dimensional plot in which the response variable, log(degradation rate), lies on a plane with a slope of E_a/R along the temperature axis and a slope of *B* along the RH axis, showing how E_a term is a "temperature sensitivity parameter" and *B* is a "humidity sensitivity" parameter (Figure 4.1).

It is interesting to note that no interaction term (cross term) is proposed in this model—that is, the temperature dependence of the degradation rate is independent of the RH and the humidity dependence of the degradation rate is

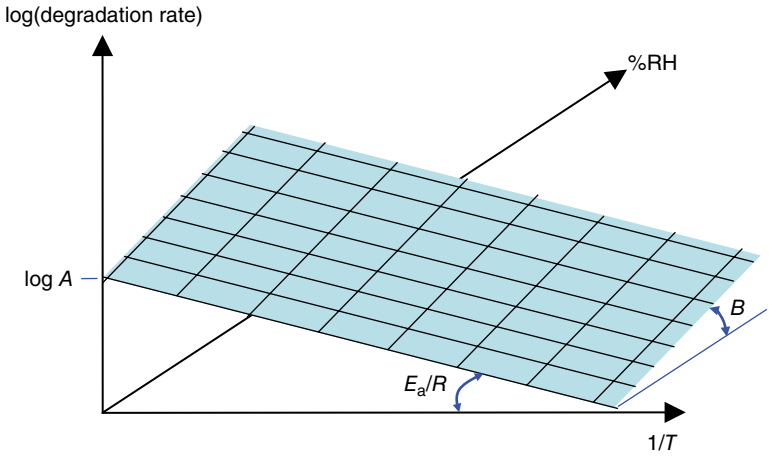


Figure 4.1 A graphical representation of Equation 4.7 in which log(degradation rate) varies linearly with 1/*T* and %RH and with no temperature–humidity interaction effects.

independent of the temperature. Studies on a number of different products have provided support for this model as shown in the following case study.

4.2.4 Case Study

In the following case study from our own laboratories, drug product A was exposed to five different accelerated conditions: 80°C/40% RH, 70°C/75% RH, 70°C/10% RH, 60°C/40% RH, and 50°C/75% RH in open dishes. The degradation versus time curve at each condition was observed to be linear over the levels of degradation obtained in this study, and so degradation rates for the different conditions were simply obtained from the slopes of those curves. Figure 4.2 shows the plot obtained from this study, where the Arrhenius plot obtained at 75% RH is essentially parallel to that obtained at 40% RH (i.e., the effect of temperature appears to be independent of RH). Thus Equation 4.7 appears to be applicable to "drug product A," without the need for a temperature–humidity interaction term (cross term), since the need for an interaction term would be indicated if the lines in Figure 4.2 were not parallel.

Also seen in Figure 4.2 is that the vertical distance between these lines appears to be approximately proportional to the difference in RH; this is better seen in Figure 4.3 obtained from the same raw data, in which log(degradation rate) appears to be linearly proportional to the RH. Note that the slope of the line in Figure 4.3 provides a means of estimating *B*, the humidity sensitivity parameter from Equation 4.7—the steeper the slope, the higher the humidity sensitivity.

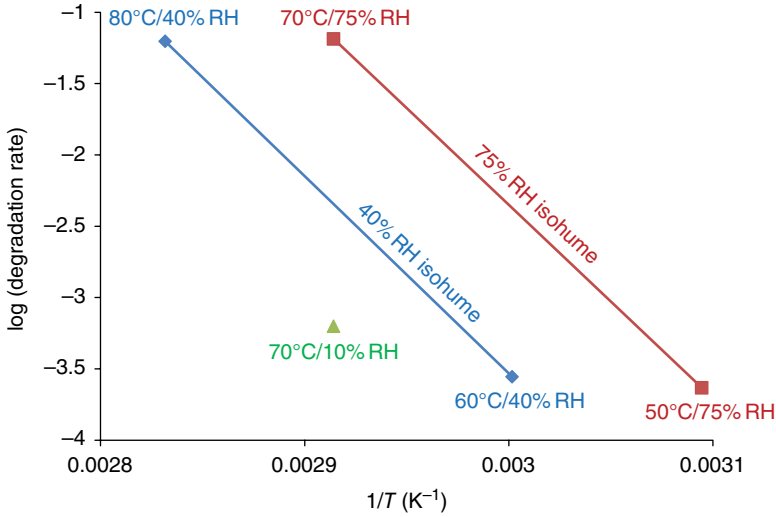


Figure 4.2 Arrhenius plot obtained for drug product A in which the effect of temperature appears to be essentially independent of relative humidity and log *k* appears to increase proportionally with relative humidity.

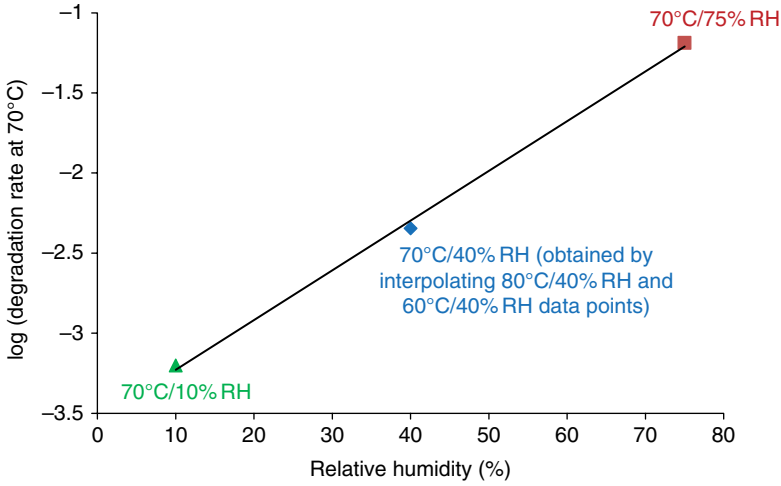


Figure 4.3 Log *k* versus relative humidity for product A, obtained at constant temperature (70°C), in which log(degradation rate) appears to be linearly proportional to the relative humidity.

4.2.4.1 Alternative Temperature–Humidity Models

The dependence of degradation rate on the combined effects of temperature and humidity described previously, summarized in Equations 4.6 and 4.7, represents perhaps the most commonly applied and simplest model for estimating degradation rate as a function of temperature and humidity. However, this model is by no means universal, and the applicability of this model for each specific pharmaceutical system needs to be demonstrated before it can be applied. Some of the possible reasons for the non-applicability of this model have already been briefly discussed in Section 4.2.1; in our experience changes in physical state (such as melting or deliquescence) as a result of changing the temperature or humidity level or the occurrence of multiple degradation processes (either physical or chemical processes) are the most common reasons for poor "fit-to-model." However, alternative temperature–humidity models have been proposed, some of which are discussed in the following text.

4.2.4.1.1 Temperature–Humidity Interaction Term and Other Model-Fitting Parameters

Equation 4.7 can be described as a "linear model," because the response variable, log(degradation rate), varies linearly according to the input variables: reciprocal temperature and RH. Linear models are often expressed in the form shown in Equation 4.8, where y is log(degradation rate) and the β parameters are model-fitting parameters—β_0 is log A (intercept), β_1 is E_a/R, and β_2 is B:

$$y = \beta_0 + \beta_1\left(\frac{1}{T}\right) + \beta_2\left(\mathrm{RH}\right) \tag{4.8}$$

This linear model may be extended to include β_{12}, a temperature–humidity interaction term, and β_{11} and β_{22} terms that allow for a nonlinear (quadratic) relationship between log(degradation rate) and $(1/T)$ and RH, as shown in Equation 4.9:

$$y = \beta_0 + \beta_1\left(\frac{1}{T}\right) + \beta_2\left(\mathrm{RH}\right) + \beta_{12}\left(\frac{1}{T}\times\mathrm{RH}\right) + \beta_{11}\left(\frac{1}{T}\right)^2 + \beta_{22}\left(\mathrm{RH}\right)^2 \tag{4.9}$$

The introduction of additional model-fitting parameters as shown in Equation 4.9 will inevitably bring about a better "fit" because more terms are available to describe the response surface—instead of a simple "plane" as shown in Figure 4.1, the response surface becomes a complex curve. However, the more parameters (β) that are introduced, the more stability conditions are required to determine those parameters; also the introduction of too many parameters can lead to "over-fitting," where the additional parameters in the model merely lead to fitting experimental "noise," and thus the predictive ability of the model decreases. Methods for avoiding over-fitting have been developed such as

"Akaike's information criterion" (AIC) and "Bayesian information criterion" (BIC) [12, 13]. Over-fitting is particularly problematic for predictions involving extrapolation of the model beyond the confines of the input data. For example, using the Arrhenius equation to predict the rate of degradation under long-term storage conditions on the basis of short-term accelerated stability data requires extrapolation of the Arrhenius plot from low values of $(1/T)$ to higher values of $(1/T)$; thus the introduction of additional terms is likely to lead to less accurate shelf-life predictions for purely *accelerated* stability studies of that type. The freedom to introduce additional terms has been reported to increase the predictive accuracy for approaches in which log(degradation rate) is predicted by *interpolation* within the confines of the experimental design of the stability protocol [14]. However, avoiding extrapolation in the $(1/T)$ axis may result in degradation levels below the LOQ (under the mild conditions) unless the stability study is run out to long time periods. The inclusion of very low degradation data into the model may be problematic where degradation versus time is not linear—that is, the initial degradation rate may not be representative of the rate over the range up to the specification level.

4.2.4.1.2 Humidity "Power" Models

As discussed in Section 4.2.2, it has also been proposed that degradation rate may be proportional to the humidity raised to the power of a constant [8]:

$$\text{Degradation rate} = A \times \exp\left(\frac{-E_a}{R.T}\right) \times H^B \tag{4.10}$$

$$\log\left(\text{degradation rate}\right) = \log A - \frac{E_a}{R.T} + B.\log\left(H\right) \tag{4.11}$$

where H is a measure of humidity, such as RH, absolute humidity, or vapor pressure; with this model, the choice of which humidity of these descriptors is used is likely to be unimportant since vapor pressure and absolute humidity are linearly correlated and

$$\text{Vapour pressure}\left(\text{VP}\right) = \text{relative humidity}\left(\text{RH}\right) \times \text{saturated vapour pressure}\left(\text{SVP}\right) \tag{4.12}$$

$$\text{SVP} = \text{constant}_1 \times \exp\left[\text{constant}_2 \times \left(\frac{1}{T}\right)\right] \tag{4.13}$$

The mathematical consequence of this is that if the degradation rate is dependent on vapor pressure as shown in Equation 4.14, then it is also dependent on RH as shown in Equation 4.15, which is obtained by substituting Equations 4.12 and 4.13 into Equation 4.14:

$$\log\left(\text{degradation rate}\right) = \log A - \left(\frac{E_a}{R}\right)\left(\frac{1}{T}\right) + B.\log\left(\text{VP}\right) \tag{4.14}$$

$$\log\left(\text{degradation rate}\right) = \log\left(A \times \text{constant}_1^{B}\right) - \left(\frac{\left(E_a + B \times \text{constant}_2\right)}{R}\right)\left(\frac{1}{T}\right) + B.\log\left(\text{RH}\right) \tag{4.15}$$

Equations 4.14 and 4.15 are of the same structure, that is:

$$\log\left(\text{degradation rate}\right) = \text{constant}_a - \text{constant}_b\left(\frac{1}{T}\right) + \text{constant}_c \times \log\left(\text{RH}\right)$$

This means that vapor pressure (absolute humidity) and RH are virtually equivalent for the purposes of modeling the combined effects of temperature and humidity based on a "humidity power" model (although absolute humidity and RH do not result in *exactly* the same model, one humidity expression will inevitably lead to a better model fit than the other).

The exponential humidity model (Equation 4.7) has been applied more widely than the humidity power model (Equation 4.11), but it is disputed which model generally fits experimental data better—a complicating factor is that the humidity power model is typically applied in combination with an additional "degradation shape" parameter (discussed in more detail in Section 4.4.1.2), which has thus far prevented a like-for-like comparison with the exponential model. However, our investigations in this area appear to support Equation 4.7, and linear relationships between log(degradation rate) and humidity (as suggested in Equation 4.7) are often reported in the literature [15] as shown, for example, in Figures 4.3 and 4.4, taken from Duncan et al. [10], whereas Equation 4.11 would suggest a linear relationship between log(degradation rate) and log(water vapor pressure).

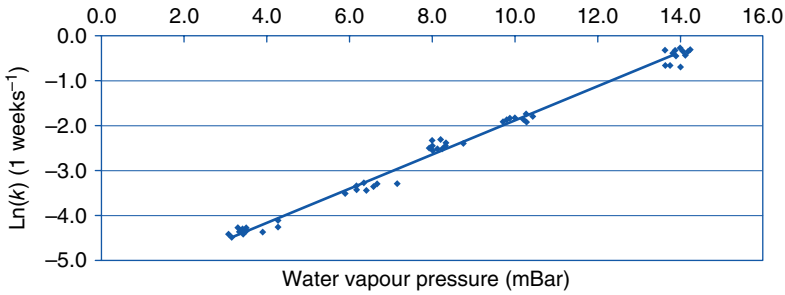


Figure 4.4 Log *k* versus relative humidity obtained at constant temperature (60°C), in which log(degradation rate) appears to be linearly proportional to the water vapor pressure. *Source:* Duncan et al. [10]. Reproduced with permission of LIGHTHOUSE.

4.2.5 Oxygen Level

There are fewer examples of studies on the effect of oxygen level on shelf life of pharmaceuticals. This may reflect that atmospheric oxygen levels are not climatically variable unlike temperature and humidity. However there are examples of pharmaceutical products that are packaged with a nitrogen-filled headspace or with an oxygen scavenger in order to improve the shelf life by reducing the rate of oxidative degradation. Studies on the benefits of reduced oxygen headspace have mainly been to support proprietary clinical or commercial products included in regulatory submissions and as such are not typically in the public domain. Also, the data interpretation in regulatory submissions would not typically attempt to quantitatively model the effect of oxygen headspace levels on the rate of degradation. A non-pharmaceutical investigation into the effect of oxygen level on the rate of oxidation proposed that log(degradation rate) is linearly proportional to log(oxygen level) [16]. A pharmaceutical example from our own laboratory found that this relationship worked reasonably well, but we also found that a straightforward linear correlation between degradation rate and oxygen level (see Figure 4.5) performed slightly better.

4.2.6 Stability Protocols for Quantifying the Effects of Temperature and Humidity

The discussion so far in this section has described how the effects of environmental factors such as temperature and humidity can be quantified and modeled. This is consistent with the general principles stated in the parent guideline

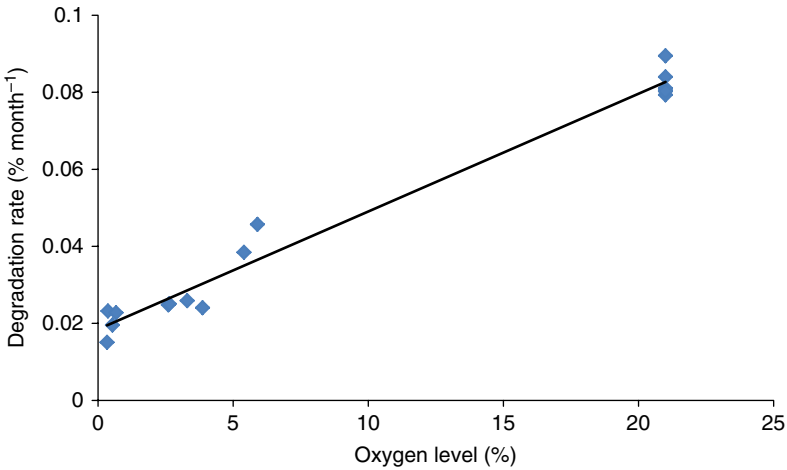


Figure 4.5 Relationship between degradation rate and oxygen headspace level for "product B."

ICH Q1A(R2): that the purpose of stability testing is to "provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, and to establish a re-test period for the drug substance or a shelf-life for the drug product and recommended storage conditions." Quantifying the effects of temperature and humidity allows the stability performance in any pack type and in any climatic zone to be predicted, which can be a significant advantage.

ICH Q1A(R2) provides clear guidance for the long-term, intermediate, and accelerated stability conditions required for registration applications. However, in order to gain a more complete understanding of how the quality of the drug substance or drug product varies with time under the influence of temperature and humidity, it can be beneficial to study stability conditions in addition to those suggested in ICH Q1A(R2).

4.2.6.1 The Case for Including Studies on Unpackaged (or Fixed Humidity) Samples

It is common to conduct stability studies only on packaged material, since ICH Q1A(R2) states that "the stability studies should be conducted on the drug substance packaged in a container closure system that is the same as or simulates the packaging proposed for storage and distribution"; for drug products, it states that "stability testing should be conducted on the dosage form packaged in the container closure system proposed for marketing." While the testing of packaged material is necessary for regulatory purposes, it can obscure the true effects of humidity (and less commonly obscure the true effects of oxygen exposure), since the environmental conditions inside the packaging are not fixed and are different to those external to the packaging. The conditions inside the packaging can be accurately simulated using well-established science (briefly described in Section 4.3.1), but stability protocols in which the humidity exposure of the actual drug substance or drug product is fixed and accurately known (such as protocols conducted on unpackaged material) are preferable for the purposes of quantifying and modeling the true effects of humidity. Conducting stability studies on unpackaged material is in line with the guideline that states that "studies carried out on the drug product outside its immediate container or in other packaging materials can form a useful part of the stress testing of the dosage form or can be considered as supporting information, respectively." Having both packaged and unpackaged (or fixed humidity) arms in stability protocols is beneficial for many situations because it enables a temperature–humidity model to be built using the unpackaged data and enables the model to be verified against the packaged data.

4.2.6.2 The Case for Water Activity Determinations of Packaged Stability Samples

The measurement of the water activity of packaged products at time points during the stability study can be extremely useful when building a detailed understanding of the effects of moisture on the stability of a product. Detailed definitions of water activity can be found in the literature [17], but for the purposes of this discussion, the water activity of the product and the RH of the headspace above the product can be considered as the same (since the water status of the product is in equilibrium with its surroundings). Therefore it can be seen how the water activity measurement relates directly to the rate of degradation (e.g., via Equation 4.7 or 4.11). Water activity determinations have advantages over water content determinations (obtained via, e.g., Karl Fischer determinations) for a number of reasons:

- Water activity determinations are nondestructive.
- Models that relate water content directly to the rate of degradation are likely to not apply to systems with containing bound water [18, 19].
- Trending analysis of stability data for water content determinations often suggests a lack of trend, which in many cases probably reflects experimental variability arising from sample handling variability prior to testing (i.e., the moisture level of the sample will equilibrate with the laboratory ambient conditions after removal from the stability chambers and during any sample manipulations such as weighing or grinding). Water activity determinations are subject to the same problems, but the sample does not generally require any manipulations prior to testing, which allows the water activity to be determined immediately for samples removed directly from the stability packaging.

4.2.6.3 Stability Protocol Design

It is important to consider the purpose of the stability study when designing the stability protocol. For example, in some situations it may be desirable to have a rapid indication of product stability and the effects of temperature and humidity; in such cases a protocol on unpackaged material comprising only a relatively small number of accelerated conditions may be warranted. However, the discussion in this section is focused on stability protocols relevant to the ICH guidelines, which are written for drug substances and drug products intended for registration application, where a high degree of confidence is required in the conclusions of the stability study.

In many circumstances, it may be warranted to conduct only the basic stability conditions stipulated in ICH Q1A(R2), but the following discussion is relevant to those wishing to build a more quantitative model for the effects of temperature and humidity. There is no "one-size-fits-all" stability protocol design; each stability protocol needs to be designed on the basis of the physical

characteristics of the individual drug substance/drug product and on the purpose of the stability study.

4.2.6.3.1 Selecting the Number of Different Storage Conditions to Be Evaluated

In order to determine how temperature and humidity affect the rate of degradation, it is necessary to conduct stability studies over multiple different stability conditions (i.e., temperature–humidity combinations). In modeling terms, the number of unknown parameters in the model determines the minimum number of stability conditions required in the study. For example, Equation 4.7 has three parameters that are required to be determined—A, E_a, and B—so a minimum of three stability conditions are required. However, having only three conditions would provide no information on how reliable the model is: it is analogous to drawing a line of best fit through two data points in a simple linear regression experiment. Therefore substantially more than three conditions should be evaluated to provide what is referred to as an "overdetermined" system. The number of stability conditions evaluated is often dependent on how much resource is available to conduct the study and the degree of reliability that is required. Evaluation of approximately 7 or 8 different conditions represents a typical compromise between resource requirements and reliability requirements. For each condition it is advisable to obtain at least two different time points in order to assess the shape of the degradation profile (discussed in Section 4.4.1). If additional terms are included (e.g., as shown in Equation 4.9), then even more stability conditions would be required.

4.2.6.3.2 Selection of Temperature–Humidity Conditions and Stability Time Points

When selecting the stability conditions, it is important that the physical state of the sample under each of the stability conditions should be the same as that under the long-term conditions. Physical changes include melts, glass transitions, deliquescence, hydrate formation, dehydrate formation, and solid-form changes. It is important to consider the combined effects of temperature and humidity; for example, higher humidity levels typically lower the glass transition temperature. Higher temperature conditions not only result in shorter stability studies but also require a larger extrapolation of the Arrhenius plot (i.e., log(degradation rate) vs. $1/T$), which could decrease the accuracy of the model if the Arrhenius plot shows any signs of nonlinearity. The inclusion of a low temperature condition (such as 5°C) generally serves as a "control" sample to allow the growth in degradation levels in other conditions to be assessed more accurately. The humidity range studied would typically span the potential "real-world" humidity exposure of packaged material; for example, 5–10% RH represents a typical lower-end RH level, and 75% RH represents a typical upper-end RH level.

When designing the stability protocol, it is also important to ensure that each stability condition has a similar influence on the model parameters; some

conditions may have significantly more influence than others depending on which temperature–humidity combinations are used in the protocol. The degree of influence may be measured, for example, by calculating the "leverage" of each stability condition or by calculating the "Cook's distance" [20]. Generally speaking, the more balanced and equally spaced the temperature and humidity conditions are, the more consistent the "leverage" is across different conditions.

From a modeling perspective, one problem encountered when designing a "balanced" stability protocol is that it can take a very long time for any observable degradation to occur at low temperature, low humidity conditions. This presents a problem because stability data analysis typically occurs at an interim period before the conclusion of the stability study, and thus little or no observable degradation has been obtained at that point. This results either in an absence of data or in very low levels of degradation under those conditions. The problem with an absence of data is obvious: there are fewer data points on which to base the model, and it will affect the "balance" of the stability protocol design. The problem with very low levels of degradation is less obvious. Firstly there is less precision in the measurement, and in many situations, the stability profile is not linear, that is, the initial rate of degradation can be very different from that observed later, which can bias the estimation of the degradation rate relevant for estimating the expiry date (i.e., the time taken for the attribute to breach the specification limit); this is discussed in detail in Section 4.4.1. In order to reduce this "bias," the time points tested for accelerated conditions should be short relative to the milder conditions so that approximately the same extent of degradation is obtained across different stability conditions. This is has been referred to as an "isoconversion" approach (and is covered in more detail in Section 4.4.1.1) [3].

Interim analysis of stability data (i.e., setting expiry periods prior to the full completion of the stability study) inevitably requires extrapolation of one sort or another: mild conditions require extrapolation in the "degradation versus time" space (up to the specification level), and accelerated conditions require extrapolation in the "degradation versus temperature or humidity" space to long-term conditions. There has been some debate regarding which is safer: avoiding low degradation or avoiding harsher conditions, but here we have drawn attention to the fact that there are assumptions and limitations with both approaches, and including elements from both into the stability protocol design can improve understanding and increase confidence when setting expiry periods.

4.2.6.3.3 Practical Considerations for Fixed Humidity Stability Studies

The control of temperature and humidity for stability samples can be achieved with humidity-controlled ovens; ovens set at 25°C/60% RH, 30°C/65% RH, 30°C/75% RH, and 40°C/75% RH are common since these are the conditions

cited in ICH Q1A(R2). Ovens set at 30°C/35% RH are also common since this is the recommended "hot, dry" storage condition for climatic zone III [21, 22]. Storage at other fixed temperature–humidity conditions may present a problem since investment is required for additional humidity-controlled ovens, although the increased focus on "predictive stability" has resulted in the major pharmaceutical companies making that investment.

A range of fixed temperature–humidity conditions can be achieved using non-humidity-controlled ovens and airtight containers. Humidity control inside the airtight containers can be achieved, for example, by pre-equilibrating the samples to the desired humidity level prior to storage or by using commercially available or saturated salt solutions to maintain the desired humidity level [23]. For example, a saturated solution of magnesium chloride maintains an RH of approximately 30.5% RH at 50°C (the humidity level changes slightly as the temperature is varied); similarly saturated sodium bromide maintains a level of approximately 50.9% RH at 50°C. If airtight containers are used, it is recommended to monitor the internal humidity; small battery-operated temperature–humidity probes are commercially available for this purpose.

4.2.7 Typical Values for Temperature and Humidity Sensitivity of Pharmaceutical Products

Figure 4.6 shows the humidity and temperature sensitivity of about 60 products determined in our laboratories. The humidity sensitivity is measured in terms of the B value, and the temperature sensitivity is measured in terms of activation energy, E_a, as described previously and based on Equation 4.7. As can be seen, the average activation energy is about 120 kJ.mol^{-1}, and the average B value is about 0.04 (%$^{-1}$), although a wide range is observed for both E_a and B. This suggests that for a "typical" product with an E_a value of 120 kJ.mol^{-1}, there would be about a 10-fold acceleration going from the long-term temperature (25°C) to the accelerated temperature (40°C) and for a refrigerated product about a 34-fold acceleration going from the long-term to the accelerated temperature (5–25°C). Therefore no significant change at accelerated condition with 6 months as stipulated in ICH Q1E represents a significant safety margin for most products—particularly for refrigerated products, since 6 months at the accelerated condition will on average equate to about 60 months for a product intended for storage at 25°C/60% RH and about 200 months for a product intended for storage at 5°C. However, for refrigerated products the decision tree in ICH Q1E also entails an assessment of accelerated data after 3 months, which equates to about 100 months under the long-term conditions.

The average B value of about 0.04 (%$^{-1}$) equates to a doubling of reaction rate for every 20% RH increase. The wide range of E_a and B values seen in Figure 4.6 highlights the difficulty in applying any rule of thumb for assessing accelerated

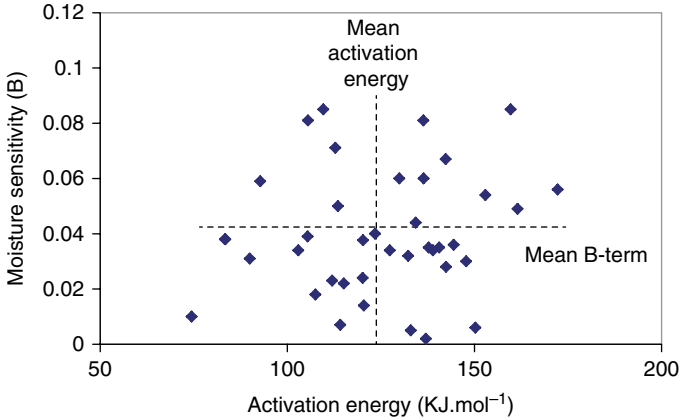


Figure 4.6 The humidity and temperature sensitivity of about 60 products.

data and provides an argument for determining the temperature and humidity sensitivity on a case-by-case basis.

4.3 Multifactor Stability Studies

The parent guideline ICH Q1A(R2) states that stability studies should be conducted on at least three batches, and for drug product, this should be performed on each individual strength and packaging type unless bracketing or matrixing is applied. If factors such as strength and packaging do not appear to affect stability performance, then the stability data can be pooled to provide a shelf life that is applicable to all strengths and pack types. However, for some products, such factors can affect stability performance; ICH Q1E states that where there are differences are readily attributed to a particular factor, different shelf lives can be assigned, and a discussion should be provided to address the cause for the differences. This section provides a brief discussion on the possible effects of these factors on product stability.

4.3.1 The Effect of Packaging Configuration on Stability Performance

Packaging provides a barrier to the external conditions including factors such as humidity, oxygen, and light; therefore the environmental conditions inside packaging can be very different to the storage conditions exterior to the packaging. So, for example, if a packaged product is stored in a 25°C/60% RH chamber, the storage temperature of the product can be assumed to be 25°C, but the precise humidity exposure of the product cannot be known from this information alone. In order to know the humidity conditions inside the packaging, this needs to be measured but can also be calculated if the water vapor

transmission rate (WVTR) of the packaging, the moisture adsorptive properties of the contents of the packaging (i.e., the product itself and any desiccants that may be present), and the water activity of the contents at the time of packaging are known [24, 25].

WVTR is a measure of the protection from moisture provided by the packaging: the higher the WVTR, the more rapid the water permeation. The WVTR increases proportionally to the difference between the internal and external RH conditions as shown in Equation 4.16:

$$\mathrm{WVTR} = P \times (\mathrm{RH}_{\mathrm{ext}} - \mathrm{RH}_{\mathrm{int}}) \tag{4.16}$$

where P is a permeability constant and $\mathrm{RH}_{\mathrm{ext}}$ and $\mathrm{RH}_{\mathrm{int}}$ are the external and internal RH conditions, respectively. Permeability, P, varies with temperature according to Equation 4.2.

The moisture adsorptive properties of materials are typically described in terms of their moisture vapor sorption isotherms, which show the relationship between the water content of a material (e.g., in % w/w) and the ambient RH. Moisture-absorptive materials and large amounts of materials inside packaging help to "buffer" the effects of water ingress—that is, this slows down the rate of change in RH inside the packaging.

These effects can be seen in Figure 4.7, which compares the humidity inside packaging for three different scenarios: the rate of change is fastest in scenarios with the most permeable packaging or with the least moisture-absorptive contents. If product and strength are kept the same, then packaging configurations with the same "WVTR per unit" will have the same RH versus time curve; it is for this reason that "WVTR per unit" is an acceptable basis for bracketing stability studies for a given product strength. "WVTR per unit" is not suitable when comparing the moisture protection across different product strengths or when desiccants are used (as discussed later).

4.3.1.1 Desiccants

The addition of desiccants to the packaging is potentially beneficial for moisture-sensitive products, not only because they dry out the product initially but also because they buffer against changes in humidity because of their large water containing capacity (i.e., the steepness of their water sorption isotherm). However, the addition of desiccants also makes it more complicated to quantitatively link the stability behavior across different packaging configurations since simple concepts such as "WVTR per unit" no longer apply (unless product/desiccant mass ratios are maintained). By way of example Figure 4.8 shows how the humidity in packaging changes over time for different bottle counts, each packaged with 1 g canister of silica desiccant; Figure 4.8 shows how the simple "WVTR per unit" is no longer useful in establishing which configuration is worst case (and hence no longer a useful basis for a bracketing strategy),

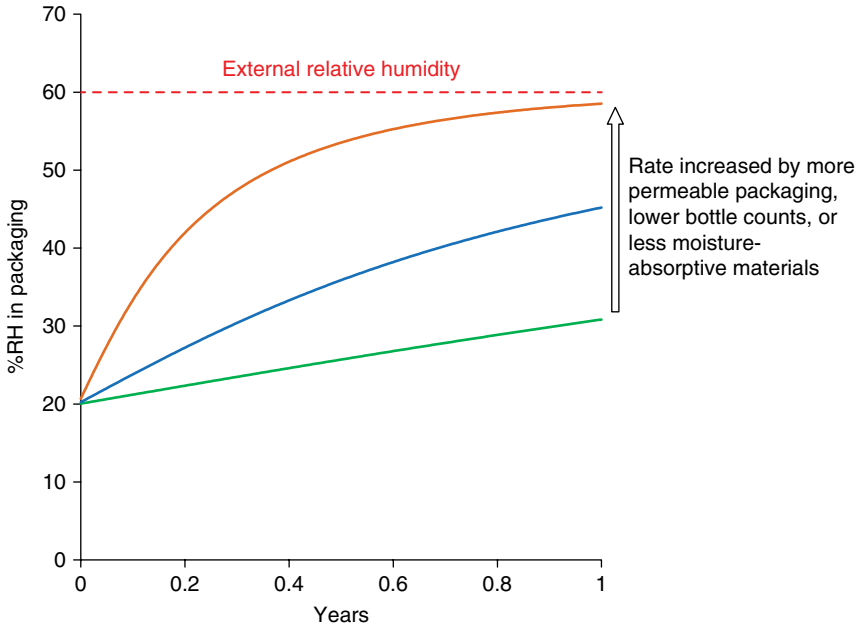


Figure 4.7 The rate of change of the humidity inside packaging depends on packaging permeability, the moisture sorption properties, and the amount of the material inside the packaging.

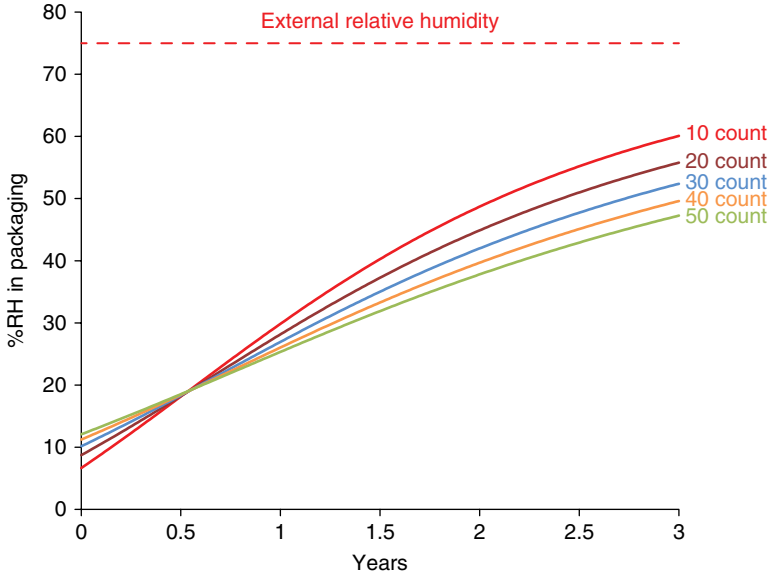


Figure 4.8 The humidity inside 60 cc HDPE bottles, containing a 1 g silica desiccant and variable numbers of tablets.

since low count bottles provide drier conditions at early time points but provide higher humidity conditions at later time points. Therefore careful consideration is required if a bracketing strategy is pursued for a product requiring desiccants.

4.3.2 The Effect of Product Strength on Stability Performance

Product strength can have a significant effect on stability performance; the precise nature of this effect depends on whether or not the different strengths are made from a "common blend." Different strengths are said to have a "common blend" if they share the same qualitative and quantitative composition, that is, all the ingredients are added in the same ratios; a "non-common blend" usually refers to different strengths that have the same qualitative composition but different quantitative composition, that is, they have the same ingredients, but the ingredients are present at different ratios. The parent guideline ICH Q1A(R2) states that bracketing is applicable if the strengths are identical or very closely related in composition (i.e., made from a common blend), and ICH Q1E states that a discussion should be provided to address the cause for the differences between strengths. The following is a brief discussion on the scientific basis for possible differences in stability performance between different strengths.

4.3.2.1 Common Blend

Products that share a common blend are likely to exhibit very similar stability performance, although differences in dissolution performance are perhaps most likely to be evident because differences in factors such as hydrodynamics, tablet hardness, and lower strengths will inevitably have better "sink" conditions. In terms of chemical degradation, higher strength tablets tend to be manufactured using higher compression forces during tableting, which could affect the rate of degradation. However, the following case study provides an example where different strengths exhibit consistent degradation behavior when stored under the same conditions but exhibit different stability behaviors when packaged because of different humidity levels inside the packaging.

4.3.2.2 Common Blend Case Study

An accelerated stability study was conducted on three different strengths of "product D" (15, 30, and 45 mg strengths) made from a common blend. During the stability study, the tablets were stored in open glass petri dishes, and the stability conditions are shown in Table 4.1. At each condition, the degradation levels obtained for the three strengths were within experimental error of each other, indicating that the three different strengths perform very similarly with respect to chemical degradation; this can also be seen in Figure 4.9 in which the raw data has been processed into log(degradation rate) data and plotted against

Table 4.1 Accelerated stability data obtained for "product D."

Duration (days)	Temperature (°C)	Humidity (%RH)	Degradation growth (%w/w)		
			15 mg strength	30 mg strength	45 mg strength
14	50	75	0.29%	0.28%	0.27%
14	60	40	0.26%	0.23%	0.22%
14	70	10	0.29%	0.29%	0.27%
2	70	75	0.39%	0.40%	0.40%
2	80	40	0.54%	0.48%	0.44%

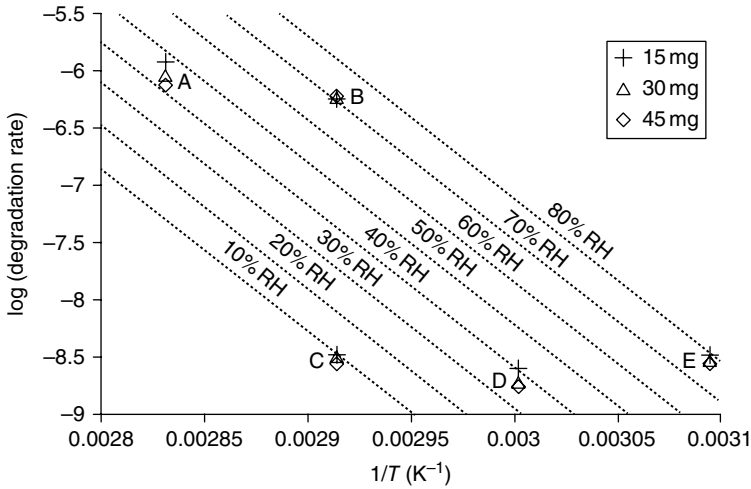


Figure 4.9 Accelerated stability data from product D (Table 4.1) presented as an Arrhenius plot. The points clustered at A are 80°C/40% RH data, B are 70°C/75% RH data, C are 70°C/10% RH data, D are 60°C/40% RH data, and E are 50°C/75% RH data. The dotted lines are the Arrhenius plots expected at different relative humidity levels based on Equation 4.7.

reciprocal temperature (i.e., presented as an "Arrhenius plot"): the stability data from the three different strengths are tightly clustered within each stability condition. Figure 4.9 also shows that the degradation data obtained from this study conform well to Equation 4.7, with E_a and B parameters of approximately 118 kJ.mol^{-1} and 0.037 (%$^{-1}$), respectively; this enables the long-term degradation behavior in packaging to be predicted with reasonable confidence. Figure 4.10 shows the predicted long-term degradation in 30-count, 60 cc HDPE bottles calculated from the accelerated stability data in Table 4.1. Despite

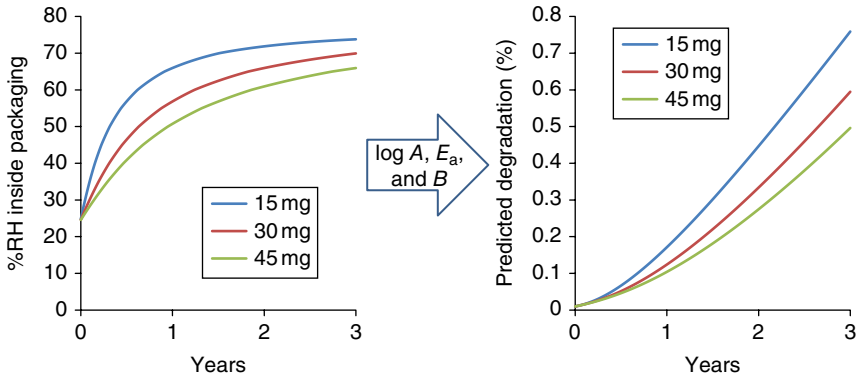


Figure 4.10 Predicted long-term degradation behavior for product D when three different strengths are packaged in 30 count HDPE bottles and stored at 30°C/75% RH. The log *A*, E_a, and *B* information from the accelerated stability study (Table 4.1 and Figure 4.11) can be used to transform the "%RH in packaging" information into degradation information.

the fact that all three strengths performed similarly in open glass petri dishes (Table 4.1), a slight difference in stability performance is expected once packaged into 30 count bottles. This is because the bottles containing the 15 mg strength tablets have in effect half the amount of product compared with the bottles containing the 30 mg strength tablets and a third of the amount of product compared with the bottles containing the 45 mg strength tablets: greater amounts of product slow down the rate of humidity increase inside the bottles, and the *B* value for this product ($0.037\%^{-1}$) indicates this is a moderately moisture-sensitive product. If "WVTR per mass of product" were kept constant across the three different product strengths (as opposed to "WVTR per unit"), then equivalent stability performance in packaging would be expected.

The presentation of stability predictions, such as that shown in Figure 4.10 in regulatory filings alongside long-term data that demonstrates the accuracy of the prediction, would provide a powerful means of demonstrating a thorough understanding of the effects of factors such as strength, packaging type and fill, temperature, and humidity in line with the aims of ICH Q1E.

4.3.2.3 Non-common Blend

ICH Q1D states that bracketing can be applied—with justification—to studies with multiple strengths where the relative amounts of drug substance and excipients change in a formulation. However, if stability trends are observed in such a study (i.e., one comprising multiple strengths made with the same excipients but in different proportions, i.e., have the same *qualitative* composition, but have different *quantitative* composition), then it is likely that the different strengths will exhibit different *rates* in those stability trends. For example, product strengths with higher excipient/API ratios tend to exhibit more rapid

chemical degradation than those with lower excipient/API ratios (when all other factors are kept constant). This is exemplified in the following study in which the drug load in simple API/excipient binary blends was observed to affect the rate of degradation.

4.3.2.3.1 Studies into the Effect of Drug Load (API/Excipient Ratio)

The rate of degradation of API "E" was studied at multiple different API loads (%w/w) in binary mixtures with microcrystalline cellulose; the binary mixtures were prepared by simple mixing (tumbling). The rate of degradation was studied under accelerated conditions (80°C/40% RH) in order to reduce the timescale of the experiment, but the observations are relevant to long-term storage conditions. The extent of degradation measured after 1 month's storage is presented in Figure 4.11, which shows that lower API loadings degrade more rapidly. Moreover, it appears to be possible to model the rate of degradation according to Equation 4.17, which was derived on the basis of the theoretical contact surface area between the API and the excipient.

In Equation 4.17, API load is expressed as a number between 0 (infinitely dilute) and 1 (pure API); $\%Deg_{API}$ is the % degradation observed for API in the absence of excipient; $\%Deg_{Max}$ is a constant obtained by fitting. It represents the theoretical % degradation at an infinitely dilute API loading, and K is

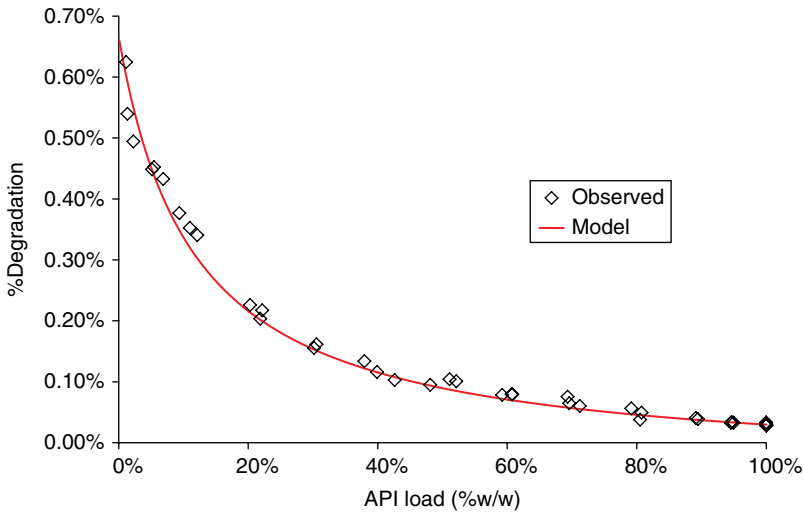


Figure 4.11 Extent of degradation observed after 1 month's storage at 80°C/40% RH when API "E" is mixed with microcrystalline cellulose in different ratios; approximately 40 different mixtures were measured. The curve-labeled "model" shows the levels predicted in Equation 4.17.

another constant obtained by fitting—but it is expected to be related to the ratio of the API/excipient specific surface areas ($m^2.g^{-1}$):

$$\%\text{Degradation} = \%\text{Deg}_{\text{API}} + \%\text{Deg}_{\max} . \frac{1 - \text{APIload}}{K.\left(\text{APIload}\right) + 1 - \text{APIload}} \quad (4.17)$$

In addition to API "E," two other API/excipient systems were studied using different storage conditions, and in both cases, Equation 4.17 was observed to apply, albeit with different fitting parameters.

These studies indicate that products with different strengths made from a non-common blend of excipients (e.g., with different API loadings) are likely to exhibit different rates of degradation, but these studies provide a basis for rationalizing those differences and lead to the possibility of presenting stability information in a way that encompasses all strengths, as opposed to discussing the strengths separately.

4.4 Data Evaluation and Statistical Considerations

ICH Q1E is primarily concerned with how stability data should be processed. It covers topics such as how and when stability data can be extrapolated and combined (pooled), and provides guidance on how stability studies can be bracketed and matrixed. In this section, a discussion on the shapes of degradation is provided, which is relevant to stability data extrapolation, and discussions on the "poolability" criteria and the potential effects of rounding and replicate strategy on shelf life are also included.

4.4.1 Interpreting the Shapes of Degradation Curves

According to the parent guideline ICH Q1A(R2), limited extrapolation of the real-time data from the long-term storage condition beyond the observed range to extend the retest period can be undertaken if justified on the basis of what is known about the mechanism of degradation, while ICH Q1E acknowledges that the correctness of the assumed change pattern is critical when extrapolation is considered. If the degradation versus time curve is essentially linear, then this facilitates statistical evaluations such as regression and extrapolation. In our experiences, degradation curves can be considered linear in about 50% of cases for solid products. If the relationship is nonlinear, then the guidelines suggest that the data can be linearized using quadratic, cubic, and/or logarithmic transformations. There are a number of possible causes of

nonlinear degradation behavior, particularly in the solid state, some of which are listed as follows:

- Complex chemical degradation reaction scheme. The observed degradation products may be part of a complicated reaction scheme involving multiple consecutive, competing, and reversible steps.
- Heterogeneous environment. In the solid state, there could be a number of, or indeed a continuum of, different physical environments of the API. Each environment could exhibit a different susceptibility to degradation. Also the physical state of the sample may also change over time, resulting in different proportions of reactive environments as time progresses.
- The limit of degradation, "Deg_{lim} or Deg_{∞}," is unknown and is <<100%. Related to the previous bullet, only a small proportion of API may be in a reactive environment. For example, a situation where only API in an amorphous state exhibits any appreciable degradation and only 1% of the API is present in the amorphous state, the observed degradation would exhibit a curve as it approaches its limit of 1% degradation (i.e., "Deg_{lim}" is 1%).
- Nonlinear physical and solid-state processes. In the solid state, it is possible that degradation could proceed via nonlinear kinetics as a result of, for example, nucleation, diffusion, and contracting volume or contracting area processes (some examples are provided in Table 4.2) [26].
- Non-fixed humidity environment. As already discussed in Section 4.3.1, stability studies of packaged products inevitably result in a non-fixed

Table 4.2 Kinetic expressions for some common solid-state degradation processes.

Solid-state degradation process	$\%Deg_t =$	$\%Deg_t$ is proportional to t^n (when $\%Deg_t << \%Deg_\infty$), where:
Zero order	kt	$n = 1$
First order	$[\%Deg]_\infty \left(1 - e^{-kt}\right)$	$n = 1$
Second order	$[\%Deg]_\infty \left(1 - \frac{1}{1+kt}\right)$	$n = 1$
Nucleation, Avrami–Erofeev (order 2)	$[\%Deg]_\infty \left(1 - e^{-(kt)^2}\right)$	$n = 2$
Nucleation, Avrami–Erofeev (order 3)	$[\%Deg]_\infty \left(1 - e^{-(kt)^3}\right)$	$n = 3$
Contracting area	$[\%Deg]_\infty \left[1 - (1 - kt)^2\right]$	$n = 1$
Contracting volume	$[\%Deg]_\infty \left[1 - (1 - kt)^3\right]$	$n = 1$
3-D diffusion	$[\%Deg]_\infty \left[1 - \left(1 - (kt)^{1/2}\right)^3\right]$	$n = 0.5$

humidity environment for the product: the humidity inside the packaging will typically slowly increase toward that of the external environment as a result of moisture permeation through the packaging. As can be seen in Figure 4.6, the rate of degradation for most products is significantly affected by the humidity environment, but as discussed in Section 4.2.6.1, this cause of nonlinear degradation can be eliminated by conducting stability studies on fixed humidity (e.g., unpackaged) samples.

Any real-world nonlinear degradation could arise from any combination of the causes listed and not listed previously. Therefore it is very difficult in practice to assign *a priori* a linear transformation based only on an understanding of the underlying mechanisms: most if not all historical linear transformations of solid-state packaged stability data used in regulatory filings will be empirical in nature to some extent. However, the purpose of this brief section is to suggest how linear transformations might be more mechanistically based in the future.

4.4.1.1 "Isoconversion"

Isoconversion methods allow nonlinear degradation rates obtained in different situations to be compared without the need for expressing the degradation curve as a rate equation. The time taken to reach a certain level of degradation (i.e., the same level of conversion, hence "isoconversion") is used as the measure of the degradation rate. With isoconversion methods, it does not matter what the shape of the degradation curve is, as long as that shape is consistent across all the different situations. Note that in this context, the *shape* of the curve is considered separately from the *timescale* of the curve. This distinction can be seen in Figure 4.12a in which degradation curves obtained at 50°C/75% RH, 60°C/75% RH, and 70°C/75% RH are shown. The curves clearly have different rates, but as shown in Figure 4.12b, the shape of the curve remains the same under each condition when the curves are plotted using a scaled time axis. Therefore it is possible to state that regardless of the cause of the nonlinearity of the curves in Figure 4.12, the rate of degradation at 70°C/75% RH is 3.2 times faster than at 60°C/75% RH and 11.2 times faster than at 50°C/75% RH. Information such as this can be used to calculate the temperature and humidity sensitivity for products, even if they have non-defined nonlinear degradation behavior. In this example the 3.2- and 11.2-fold acceleration equates to an activation energy of 111.3 $kJ.mol^{-1}$ as calculated using the Arrhenius equation (Equation 4.1). In cases such as this, it may be possible to obtain an overarching quantitative understanding of the stability behavior of the material that encompasses all stability scenarios. Cases in which the shape of the curve differs between different situations, such as that shown in Figure 4.13, are more difficult to accurately link the stability behavior across different situations, and it may be possible only to trend the stability data for each situation in isolation from the others.

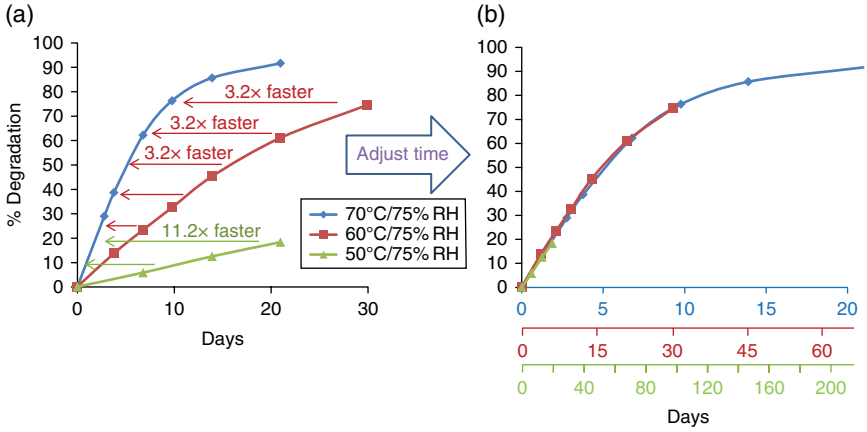


Figure 4.12 Degradation versus time curves obtained for product F at different temperatures, but under constant relative humidity. (a) The raw data. (b) The same data but with the time axis adjusted showing that the data from different conditions lie on the same overall shaped curve.

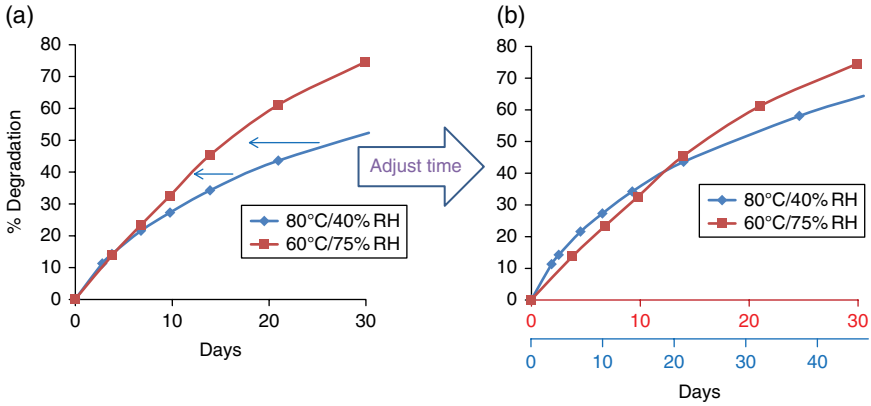


Figure 4.13 Degradation versus time curves obtained for product F under different temperature and humidity conditions. (a) The raw data. (b) The same data but with the time axis adjusted showing that the data from different conditions do not lie on the same overall shaped curve.

4.4.1.2 "Shape Parameter" Approach

Nonlinear stability trends are sometimes described on the basis of a "shape parameter" (n), in which a stability-related attribute is proportional to the stability time point raised to the power of n, as shown in Equation 4.18:

$$\text{Stability related attribute} \propto \text{time}^{n} \tag{4.18}$$

This is consistent with ICH Q1E, which suggests that the data can be linearized using a logarithmic transformation, since taking a logarithm of Equation 4.18 results in a linear relationship (Equation 4.19):

$$\log(\text{stability related attribute}) = n.\log(\text{time}) + \text{constant} \tag{4.19}$$

This approach is also somewhat consistent with kinetic expressions for many of solid-state degradation processes (listed in Table 4.2) [22]. When the extent of degradation ($\%Deg_t$) remains much lower than "$\%Deg_\infty$" (the asymptotic maximum extent of degradation), the kinetic expression for the solid-state degradation process simplifies to an expression of the form of Equation 4.18. For example, simple zero-, first- or second-order mechanisms and geometric contraction models may be approximated by Equation 4.18 with $n = 1$, diffusional mechanisms may be approximated by $n = 0.5$, and nucleation models (such as Avrami–Erofeev) may be approximated by $n = 2$ or higher, when the extent of degradation is low. In most stability studies, the extent of degradation will remain "low" since specification levels are typically less than 5%, which is likely to be much lower than the maximum possible extent of degradation under long-term or accelerated conditions.

The shape parameter approach has a potential advantage over isoconversion methods in situations where the shape of the curve is not the same under different stability situations: it may be possible to derive an expression for how the shape parameter varies according to factors such as temperature and humidity. However, this is in effect introducing more fitting parameters to the model, and therefore much more input data is required to build a reliable model, and even then it would probably be unwise to extrapolate beyond the confines of the experimental space of the input data. The shape parameter approach has the disadvantage that many "real-world" degradation curves are not well described in Equation 4.18.

4.4.1.3 Nonlinear Degradation Resulting from Non-fixed Humidity Conditions

As already discussed, the humidity conditions inside packaging is typically not fixed as a result of moisture permeating through the packaging; this can result in nonlinear degradation when the rate of degradation is sensitive to moisture. This can convolute attempts to obtain an overarching quantitative understanding of the stability behavior of the material that encompasses all pack types and stability conditions. However, the measurement of water activity, the inclusion of stability data from fixed humidity conditions, and the ability to calculate humidity levels inside packaging (discussed briefly in Section 4.3.1) can all be used to develop an overarching quantitative model of stability behavior using stability data from packaged studies.

4.4.2 Pooling Data from Multifactor Stability Studies

As previously discussed the stability of the drug product can be affected by factors such as packaging type, packaging fill, and strength; ICH Q1E states that two options can be considered when analyzing such data. The first option is to determine whether the data from all factor combinations (considered separately) support the proposed shelf life, and the second option is to determine whether the data from the different factor combinations can be combined ("pooled") to provide an overall estimate of a single shelf life. Statistical tests for "poolability" are used to determine whether this second option is appropriate. If the stability data passes the poolability tests, then the "pooled" option will generally result in a longer shelf life since the width of the confidence limits for the mean will become narrower as the amount of data increases when batches, strengths, container sizes, and/or fills are pooled. ICH Q1E recommends the use of "analysis of covariance" (ANCOVA) to test the poolability of stability data and provides high-level guidance on how to systematically assess a large amount of data obtained from a multifactor stability study. For example, the guideline recommends that poolability tests start with the slope and then the intercept of the highest order interaction and proceed to the slope and then the intercept of the simple main effects. However, the application of ANCOVA to multifactor stability studies is complex and requires decisions to be made for the hierarchical ordering of the factors; this subjectivity can lead to different shelf lives. A detailed discussion of such analyses is not provided here since such analyses are highly dependent upon the precise design of and data obtained from the multifactor stability study. Instead, the reader is referred to an excellent paper provided by Tsong et al. [27] in which a systematic, nonsubjective method for model determination and factor elimination is proposed.

A common discussion point concerning the pooling test is its power to reject the null hypothesis with the limited samples sizes common in stability studies. (N.B. Rejection of the null hypothesis indicates that the data cannot be pooled.) It was for this reason that the guidelines stipulate that poolability tests should be conducted using a significance level of 0.25 for all tests involving the batch term, instead of the more usual significance level of 0.05; this in effect makes it more difficult to pool the data and reduces the risk of the null hypothesis being not rejected due to lack of statistical power (the so-called "type I" error). This is a conservative approach since pooling leads to setting longer shelf lives. However, there are drawbacks with using a significance level of 0.25; for instance, in cases where there is no genuine difference between batches, there is a 25% chance of failing the poolability test, and the chance of passing the tests for both slope and intercept is only 56% (75% × 75%).

There are other problems associated with the ANCOVA approach as laid out in ICH Q1E. For example, since ANCOVA compares the differences in slopes and intercepts with the overall amount of variability in the data, pooling is

more likely when the variability in the data is high, that is, there is a penalty for having good studies and methods with good accuracy and precision. Similarly there is also a penalty for having more batches in the study, since having more batches increases the likelihood of detecting differences in slopes and intercepts; this penalty is made even worse by having then to base the overall shelf life on the worst batch. Therefore there is no incentive to include additional batches in the study, but on this basis, it may be possible to justify a lower significance level for studies with more batches.

4.4.3 Miscellaneous Data Analysis Considerations

4.4.3.1 Confidence Intervals and Prediction Intervals

For quantitative attributes such as assay or degradation products, ICH Q1E recommends that the retest period or shelf life is set by determining the earliest time at which the 95% confidence limit for the mean intersects the proposed acceptance criterion; this reflects that shelf life is set as the time period we expect the population mean result to remain within specification—not the time period we expect all individual results to remain within specification. It is for this reason it is not uncommon to observe individual results that are outside the 95% confidence interval. Prediction intervals (as opposed to confidence intervals) should be used if the spread of individual results is of interest.

4.4.3.2 Replicate Testing

Confidence intervals can be narrowed and hence longer shelf lives can be achieved if the variability in the result is decreased (i.e., the precision of the measurement is increased). One way in which measurement precision can be increased is by increasing the number of replicates that comprise each test. Wherever possible, replicates should include all sources of variation; for example, repeat injections within the same assay run are not true replicates since this does not take into account variability deriving from method repeatability, run-to-run variability, laboratory-to-laboratory variability, or indeed the unit-to-unit variability in the product itself. This can be seen in the following example in which the total variability in the estimate of the mean result, σ^2_{Total}, for an assay test for tablets is estimated on the basis of intermediate precision, method repeatability, and tablet-to-tablet variability; Equation 4.20 shows how the total variability, σ^2_{Total}, can be calculated from the sum of these sources of variability:

$$\sigma^2_{\text{Total}} = \frac{\sigma^2_{\text{IP}}}{N_{\text{runs}}} + \frac{\sigma^2_{\text{method}}}{N_{\text{runs}} \times N_{\text{reps}}} + \frac{\sigma^2_{\text{product}}}{N_{\text{runs}} \times N_{\text{reps}} \times N_{\text{units}}} \tag{4.20}$$

where σ_{IP} is intermediate precision variability, σ_{method} is method repeatability variability, $\sigma_{product}$ is the unit-to-unit variability of the product, N_{runs} is the number of separate runs used to analyze the batch, N_{reps} is the number of replicates carried out in each run, and N_{units} is the number of units tested in each replicate. So, for instance, if an assay test comprises a single run in which two composite samples are tested and each of those composite samples comprise 5 units, then N_{runs}, N_{reps}, and N_{units} are 1, 2, and 5, respectively. It can be seen from Equation 4.20 that more precise estimates of the batch mean are obtained by increasing the replicates; specifically, if product variability $\sigma_{product}$ is high, then the overall variability would be improved by increasing the total number of units tested (i.e., increase $N_{runs} \times N_{reps} \times N_{units}$); if σ_{method} is high, then increasing the total number of replicates (i.e., $N_{runs} \times N_{reps}$) would be beneficial; and if σ_{IP} is high, then increasing the number of runs (N_{runs}) would be beneficial.

4.4.3.3 Rounding of Data

Another issue related to precision is the rounding of data; when data are rounded, precision in estimates is lost and in certain situations can be misleading as can be seen in the following case studies of real-life situations.

4.4.3.3.1 Case Studies

Table 4.3 shows the degradation product data obtained under the long-term conditions for product G, which has a specification limit of 0.2%. Results are typically reported rounded to the same number of decimal places as the specification, which in this case is one decimal place; however, in this case the precision of the method was suitable for reporting results to 2 decimal places. Figure 4.14 shows the plots obtained from this data; Figure 4.14a shows that the degradation data is slowly increasing with time and a shelf life of less than 20 months is appropriate, but the data rounded to 1 decimal place leads to the false conclusion that there is no stability trend and a shelf life of 24 months may be set inappropriately.

Conversely in this next case study, the inappropriate rounding of data led to an exaggeration of a stability trend. Table 4.4 shows the degradation product data obtained under the long-term conditions for product H, which again has

Table 4.3 Degradation product data obtained for product G.

	Initial	3 months	6 months	9 months	12 months
Degradation products (rounded to 2 decimal places)	0.05%	0.07%	0.09%	0.12%	0.14%
Degradation products (rounded to 1 decimal place)	0.1%	0.1%	0.1%	0.1%	0.1%

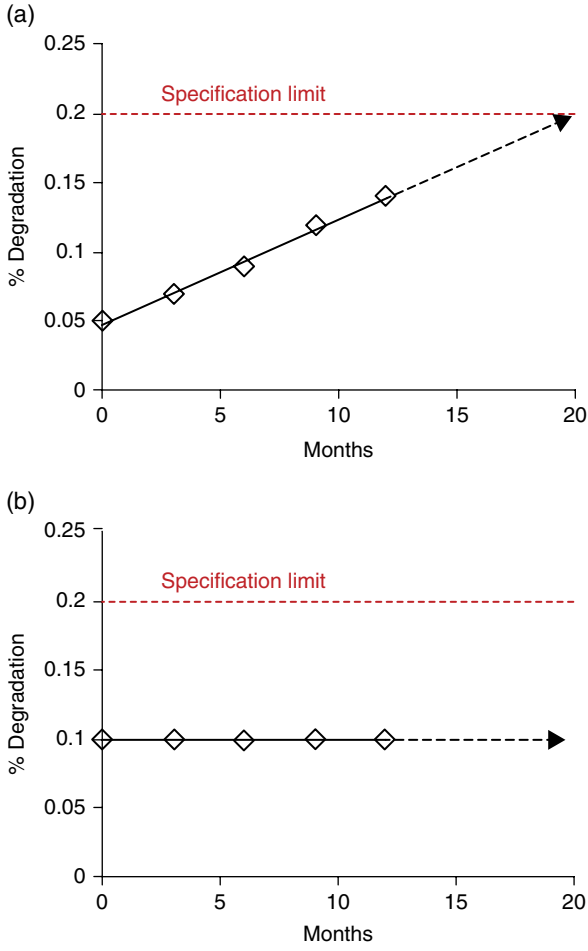


Figure 4.14 Degradation versus time curves obtained for product G. (a) The data rounded to 2 decimal places. (b) The same data rounded to 1 decimal place.

Table 4.4 Degradation product data obtained for product H.

	Initial	3 months	6 months	9 months	12 months
Degradation products (rounded to 2 decimal places)	0.14%	0.14%	0.15%	0.15%	0.15%
Degradation products (rounded to 1 decimal place)	0.1%	0.1%	0.2%	0.2%	0.2%

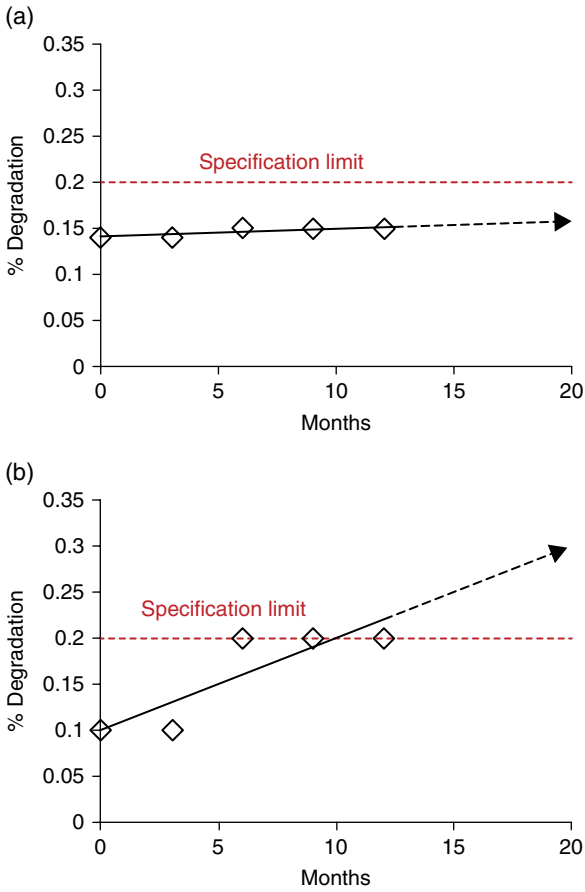


Figure 4.15 Degradation versus time curves obtained for product H. (a) The data rounded to 2 decimal places. (b) The same data rounded to 1 decimal place.

a specification limit of 0.2%, and method precision suitable for reporting results to 2 decimal places. Figure 4.15 shows how the data plotted to 1 decimal place leads to an overestimation of the stability trend.

4.5 Concluding Remarks

It is hoped that this chapter has demonstrated how, over the recent years, pharmaceutical stability data can be increasingly understood on a more quantitative, mechanistic, and scientific basis. For example, models that can relate degradation reaction rates quantitatively to environmental factors such as

temperature and humidity and packaging models that enable the humidity in packaging to be accurately simulated can be combined so that stability data can be rationalized and understood in detail. This allows data from stability studies comprising multiple storage conditions, packaging types, and packaging fills to be presented holistically and in a manner that demonstrates a thorough product understanding. Stability data from unpackaged stability studies can augment our understanding of the effects of humidity and can help to deconvolute stability data obtained from packaged material. Similarly the measurement of water activity of packaged material at any point during the stability study can, with the help of packaging simulations, help with the interpretation of the stability data. The effects of product strength have been discussed, distinguishing between strengths made from "common blend" and "non-common blend," and it appears possible to develop quantitative models that link degradation rates to product strength for both types of formulation design. An enhanced understanding of the effects of factors such as temperature, humidity, packaging, and strength is not only useful in regulatory filings for demonstrating product understanding but is also useful in a variety of risk assessment and business decision situations, such as assessing the impact of changing climatic zone, packaging, or minor formulation changes, assessing the impact of temperature and humidity excursions, and understanding risks associated with various in-use scenarios, to name but a few. Nonlinear stability trends were discussed with the intent that in the future, linear transformations of data will be increasingly based on a more mechanistic understanding of the causes of the nonlinearity.

It is worth remembering that every stability result is a scientific experiment, which is the result of various fundamental physical and chemical processes; to interpret stability data purely as a number in a solely statistical manner is a missed opportunity to gain valuable product knowledge.

References

1 ICH, Q1A(R2), 'Stability Testing of New Drug Substances and Products' (2003).
2 ICH Q1D, 'Bracketing and Matrixing Designs for Stability Testing of New Drug Substances and Products' (2002).
3 *Statistical Design and Analysis in Pharmaceutical Science: Validation, Process Controls, and Stability*, Chow, S.-C. and Liu, J., (1995) Marcel Dekker, New York.
4 ICH Q1B, 'Stability Testing: Photostability Testing of New Drug Substances and Products' (1996).
5 USP 35 General Chapter <1150>, 'Pharmaceutical Stability', pp. 763–765
6 Genton, D. and Kesselring, U.W. (1977) 'Effect of temperature and relative humidity on nitrazepam stability in solid state', *Journal of Pharmaceutical Sciences*, 66(5), pp. 676–680.

7 Waterman, K.C. and Adami, R.C. (2005) 'Accelerated aging: Prediction of chemical stability of pharmaceuticals', *International Journal of Pharmaceutics*, 293(1–2), pp. 101–125.

8 Waterman, K.C. 'Understanding and predicting pharmaceutical product shelf-life' in *Handbook of Stability Testing in Pharmaceutical Development*, Huynh-Ba, K. (Ed.) (2009), Springer Science and Business, New York, NY, pp. 115–135.

9 Waterman, K.C., Carella, A.J., Gumkowski, M.J., Lukulay, P., MacDonald, B.C., Roy, M.C. and Shamblin, S.L. (2007) 'Improved protocol and data analysis for accelerated shelf-life estimation of solid dosage forms', *Pharmaceutical Research*, 24(4), pp. 780–790.

10 Duncan, D.I., Veale, J.R., Cook, I. and Ward, K., 'White Paper on Using Laser-Based Headspace Moisture Analysis for Rapid Nondestructive Moisture Determination of Sterile Freeze-Dried Product', www.biopharma.co.uk/wp-content/uploads/2010/08/fms_whitepaper_sml.pdf (accessed April 10, 2017).

11 Clancy, D., Hodnett, N., Orr, R., Owen, M. and Peterson, J. (2016) 'Kinetic model development for accelerated stability studies', *AAPS PharmSciTech*. 10.1208/s12249-016-0565-4.

12 Akaike, H. (1974) 'A new look at the statistical model identification', *IEEE Transactions on Automatic Control*, 19, pp. 716–723.

13 Schwarz, G. (1978) 'Estimating the dimension of a model', *The Annals of Statistics*, 6, pp. 461–464

14 Regler, B., Clancy, D., Hertzler, R. and Lavrich, D., 'Predictive Stability Approaches to Assess Critical Characteristics in Drug Product Development', presentation at 2016 AAPS Annual Meeting, November, 2016, Colorado, USA.

15 Stanisz, B., Paszun, S., Strzyzycka, N. and Ptaszynski, E. (2010) 'Influence of humidity and hydroxypropyl cellulose, hydroxypropylmethyl cellulose, glyceryl behenate or magnesium stearate on the degradation kinetics of quinapril hydrochloride in solid phase', *Acta Poloniae Pharmaceutica–Drug Research*, 67, pp. 99–102.

16 Gillen, K.T., Wise, J. and Clough, R.L. (1995) 'General solution for the basic autoxidation scheme', *Polymer Degradation and Stability*, 47(1), pp. 149–161.

17 *Water Activity Applications in the Pharmaceutical Industry*, Cundell, A.M. and Fontana, A.J. (Eds.), (2009), Davis Healthcare International Publishing, LLC, River Grove, IL, 1-933722-33-9

18 Matsunaga, Y., Ohta, R., Bando, N., Yamada, H., Yuasa, H. and Kanaya, Y. (1993) 'Effects of water content on physical and chemical stability of tablets containing an anticancer drug TAT-59', *Chemical & Pharmaceutical Bulletin*, 41, pp. 720–724.

19 Guerrieri, P., Jarring, K. and Taylor, L.S. (2010) 'Impact of counterion on the chemical stability of crystalline salts of procaine', *Journal of Pharmaceutical Sciences*, 99(9), pp. 3719–3730.

20 *Generalized Linear Models, with Applications in Engineering and the Sciences*, 2nd Edition, Myers, R.H., Montgomery, D.C., Vining, G.G. and Robinson, T.J. (2010), John Wiley & Sons, Hoboken, NJ, 978-0-470-45463

21 Schumacher, P. (1972) 'Über eine für die Haltbarkeit von Arzneimitteln maßgebliche Klimaeinteilung', *Die Pharmazeutische Industrie*, 34, pp. 481–483.

22 Grimm, W. (1986) 'Storage conditions for stability testing (part 2)', *Drugs Made in Germany*, 29, pp. 39–47.

23 Greenspan, L. (1977) 'Humidity fixed points of binary saturated aqueous solutions', *Journal of Research of the National Bureau of Standards Section A: Physics and Chemistry*, 81A(1), p. 89.

24 Waterman, K.C. and Macdonald, B.C. (2010) 'Package selection for moisture protection for solid, oral drug products', *Journal of Pharmaceutical Sciences*, 99(11), pp. 4437–4452.

25 *Packaging for Climatic Protection*, Cairns, J.A., Oswin, C.R., Paine, F.A., Institute of Packaging (1974), Butterworth & Co., 0-408-00146-1.

26 Khawam, A. and Flanagan, D. R. (2006) 'Basics and applications of solid-state kinetics: a pharmaceutical perspective', *Journal of Pharmaceutical Sciences*, 95, pp. 472–498.

27 Tsong, Y., Chen, W.-J. and Chen, C.W. (2003) 'ANCOVA approach for shelf life analysis of stability study of multiple factor designs', *Journal of Biopharmaceutical Statistics*, 13, pp. 375–393.

5

Q2(R1) Validation of Analytical Procedures

Text and Methodology

Phillip Borman[1] *and David Elder*[2]

[1] *GSK, Ware, UK*
[2] *David P Elder Consultancy, Hertford, UK*

5.1 Introduction

ICH Q2(R1) [1] provides a formal overview of the criteria required to fully validate an analytical procedure. Method validation is a formal requirement of the three ICH regional regulatory agencies (FDA, EMA (European Medicines Agency), PMDA (Pharmaceutical and Medical Device Agency)) and other international and transnational agencies (e.g., BRIC (Brazil, Russia, India, China), ASEAN (Association of South East Asian States), WHO). The method validation terminology and the supporting definitions used in ICH Q2(R1) were required to "bridge the differences" that often existed between the various national pharmacopoeias, compendia, and those three regulatory agencies. While ICH Q2(R1) [1] specifically outlines those validation criteria that are applicable for a limited number of methods used for identity, assay, impurities, and limit tests for impurities, it was always the authors' intent that this guidance would cover other analytical procedures. We will explore examples of validation of other analytical methods, for example, near-infrared spectroscopy (NIR), and demonstrate that this guidance is equally applicable to non-chromatographic methodologies. ICH Q2(R1) [1] highlights that the purpose of any method validation is to demonstrate that it is "suitable for its intended purpose." This is a theme that we will continually refocus on throughout this chapter, as too often there is a slavish desire to follow the guideline without truly understanding the strengths and weaknesses of the method that has been developed. We will discuss some of the benefits of developing methods using quality by design (QbD) approaches (see ICH Q8(R2) [2]). The same QbD principles that have been developed for manufacturing processes can also be applied to ensure analytical test methods produce

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reliable data. A framework for applying QbD to analytical methods has previously been presented by Borman et al. [3], and this will be examined further within the chapter. Finally, it is important to recognize that medicinal products are developed transnationally, with products being moved across global manufacturing networks. Although some organizations still maintain their own internal global manufacturing networks, this is the exception, rather than the rule. In the majority of cases, companies utilize external service providers to manufacture and test some products within their supply chains, that is, outsourcing. In other cases, rationalization of supply chains results in products being brought back into the companies' manufacturing network, that is, in-sourcing. In all cases there is a requirement to transfer analytical methodologies between different sites. Method validation cannot be discussed in isolation of method transfer procedures, and we will devote part of this chapter to discuss this key area.

5.2 ICH Q2(R1) Validation Criteria

Analytical data should be "fit for its intended purpose," and for this to be true it must be dependable, such that any resultant decisions can be taken in confidence. Therefore, the supporting analytical validation should be viewed as the entire process of defining the analytical requirements, providing assurance that the method under consideration is fit for purpose. From a life cycle perspective, analytical method validation can be viewed as a three-stage process: (i) procedure design, (ii) performance qualification, and (iii) ongoing performance verification [4]. As the required precision is related to the use of the procedure, the need for revalidation should be assessed as part of change control (see ICH Q10 [5]). Typical changes that may require revalidation are (i) a new strength of a drug product; (ii) transfer of the procedure to a different laboratory; (iii) changes of process, resulting in new requirements for specificity; and (iv) a change in the specification (see ICH Q6A [6] and Q6B [7]). The predefined requirements for an analytical method have been defined as the analytical target profile (ATP) by an EFPIA (European Federation of Pharmaceutical Industries and Associations)/PhRMA (Pharmaceutical Research and Manufacturers of America) working group [8]. The purpose of the ATP is similar to that of the quality target product profile (QTPP) defined in ICH Q8(R2) [2], that is, to describe the predefined objectives that are required in order to apply QbD principles. In as far as it is practically possible, "the analytical procedure should be independent of the actual equipment used," with the proviso that the equipment has been adequately qualified [9]. However, it is important that this significant caveat is addressed as part of method validation, or it may significantly impact on the technology transfer process. Although not specifically stated, the statistical approaches to method validation described in ICH Q2(R1) [1] are equally applicable to small and large molecules [10]. The statistically based approach is adopted to demonstrate that the method generates data that are sufficiently accurate and precise, that is, fit for

purpose [4]. The typical validation criteria that are described within ICH Q2(R1) [1] guidance, with their definitions transcribed verbatim, together with other related definitions are listed in Table 5.1.

5.2.1 Specificity

Despite being the preferred terminology of the ICH work stream, specificity is by no means the universally accepted term within separation sciences (see Table 5.1). Indeed, selectivity has a significant number of advocates [20]. However, irrespective of preferences and definitions, there is broad-based agreement that this criterion is crucially important and is the critical foundation for any analytical procedure [9].

An assessment of the methods' specificity should be determined as part of the validation process, and the approach utilized will be dependent on the intended goal of the analytical procedure. Indeed, certain methodologies may not be specific (or not specific enough) for the intended goal, in which case orthogonal approaches using two complementary analytical procedures would be required to achieve the necessary discriminatory power. For example, titrimetric and UV potency assay for drug substance are nonspecific and cannot detect the presence of related substances, for example, process impurities or degradants, but have better precision (ca. 0.1–0.5% RSD (Residual Standard Deviation)) than the corresponding specific HPLC (High Performance Liquid Chromatography) assay methods (>0.5% RSD) [21]. Ermer [9] described how a titration assay result could be corrected to account for the related substances quantified by the complementary specific HPLC assay.

Additionally, the complex nature of many biological molecules, for example, proteins, peptides, and synthetic biomolecules, for example, oligopeptides, oligonucleotides, etc., ensures that the absolute specificity is often an unattainable goal, even using the most discriminating, sophisticated separation techniques. In these cases, complementary methodologies are the norm, as neither method has the requisite specificity to achieve the intended goal of fully characterizing the drug substance and identifying batch-to-batch differences [22]. For example, with oligonucleotides the number of closely related process impurities is substantial, and separation from the main peak is extremely challenging—if not impossible. In these cases, two or more complementary methods are typically utilized to facilitate process understanding and control. For example, anion-exchange chromatography (AEX) with denatured samples can be used to separate PO impurities, as well as differently charged impurities, whereas ion-pair reversed-phase (IP-RP) HPLC also with denatured samples can be used to separate lipophilically modified impurities, as well as shortmers ($n-1$, $n-2$, etc.) and longmers ($n+1$, $n+2$, etc.). Both these methods would be typically used with both denatured and non-denatured oligonucleotide samples [22].

For biological products specificity may need to be further defined due to the complexity of the molecules. A recent report on Transferon®, a complex

Table 5.1 Definitions provided by ICH Q2(R1) and other statutory agencies.

Validation criterion	ICH Q2(R1) definition	Other definitions/commentary
Specificity	"Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s). This definition has the following implications: Identification: to ensure the identity of an analyte. Purity tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of the impurities of an analyte, i.e. related substances test, residual solvents content, etc. Assay (content or potency): to provide an exact result, which allows an accurate statement of the content or potency of the analyte in the sample"	"The ability of a measurement procedure to determine solely the measurable quantity it purports to measure, i.e. the measurand" [11] "The ability of an assay procedure to differentiate and quantify the analyte in the presence of other components in the sample matrix" [12] "The ability to of a method to measure only what it is intended to measure" [13] "The ability to assess unequivocally the analyte in the presence of components, which may be expected to be present" [14] "The degree to which a method can quantify the analyte accurately in the presence of interferants" [15]
Selectivity	"Selectivity of an analytical method is its ability to measure accurately an analyte in the presence of interference, such as synthetic precursors, excipients, enantiomers, and known (or likely) degradation products that may be expected to be present in the sample matrix" [14]	"Other bodies, e.g. IUPAC (International Union of Pure and Applied Chemistry), AOAC (Association of Official Analytical Chemists) use the term selectivity, reserving specificity for procedures that are completely selective" [14]
Accuracy	"The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness"	"Closeness of the agreement between the result of a measurement and a true value of the measurand" [16] "The accuracy of an analytical method is the closeness of test results obtained by the method to the true value. Accuracy should be determined across its range" [4, 14]

Table 5.1 (Continued)

Validation criterion	ICH Q2(R1) definition	Other definitions/commentary
		"The closeness of mean test results obtained by method to the true value (concentration of analyte)" [12]
		"The closeness of determined value to the true value" [13]
		"The closeness of test results obtained by that method to the true value" [14]
		"Trueness is the closeness of agreement between a test result and the accepted reference value. Smaller bias means greater trueness" [15]
Precision	"The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements"	"Degree of agreement of measurements under specific conditions" [13] "Degree of agreement among individual test results when the method is applied repeatedly to multiple samples of a homogenous sample" [4, 14] "Closeness of agreement between test results obtained under stipulated conditions" [15]
Repeatability	"Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision"	"Repeatability is obtained when the analysis is carried out in one laboratory, by one operator, using one piece of equipment over a relatively short time span" [17]

(Continued)

Table 5.1 (Continued)

Validation criterion	ICH Q2(R1) definition	Other definitions/commentary
Intermediate precision	"Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc."	"Intermediate precision conditions allow for the varying of factors such as operators and apparatus over longer periods of time within a single laboratory, whereas repeatability conditions attempt to hold these factors constant" [18]
Reproducibility	"Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology)"	"Reproducibility is obtained when the analysis is carried out in more than one laboratory, by more than one operator, using more than one piece of equipment over an undefined time span" [19]
Detection limit (DL)	"The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected, but not necessarily quantitated as an exact value"	"The lowest content that can be measured with reasonable statistical certainty" [13]
	Sometimes termed limit of detection (LOD)	"Lowest amount of analyte in the sample, which can be detected, but not necessarily quantitated under stated experimental conditions" [14]
		"Smallest amount of concentration of analyte that can be reliably distinguished from zero" [13]
		"The signal (R) that is exceeded with probability α, when no analyte is present" [4]

Table 5.1 (Continued)

Validation criterion	ICH Q2(R1) definition	Other definitions/commentary
Quantitation limit (QL)	"The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products"	"Lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. Also called the LLOQ (lower limit of quantitation)" [13]
	Sometimes termed limit of quantitation (LoQ)	"The limit of quantitation is the lowest amount of analyte in the sample, which can be quantitatively determined with precision and accuracy appropriate to analyte and matrix considered" [14]
		"Lowest amount of analyte in the sample, which can be quantitatively determined with suitable precision and accuracy" [15]
Linearity	"The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample"	"Relationship between response and known concentration of analyte" [12]
		"Defines the ability of the method to obtain test results proportional to the concentration" [13]
Range	"The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity"	"Interval between the upper and lower concentration (amounts) of the analyte in the sample including the concentrations for which suitable levels of accuracy, precision and linearity have been demonstrated" [4, 14]

(Continued)

Table 5.1 (Continued)

Validation criterion	ICH Q2(R1) definition	Other definitions/commentary
Robustness	"The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage"	"The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions such as different laboratories, different analysts, different instruments, different lots of reagents, different elapsed assay times, different assay temperatures, different days, etc. Ruggedness is normally expressed as the lack of influence of operational and environmental factors of the analytical method. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from laboratory to laboratory and analyst to analyst" [14]

dialyzable leukocyte extracts (DLE), highlighted the nature of the problem. Medina-Rivero et al. [23] validated a method to characterize and release Transferon. The authors showed that Transferon generated eight main peaks (P1–P8), but other studies have reported 4, 7, or 13 main peaks for DLEs that were manufactured using similar processes. The differences were explained on the basis of differences in sensitivities of the methodologies [24].

Specificity for an identification test implies that the method, for example, IR (Infrared), UV, HPLC retention time, etc., should be able to differentiate between the analyte and any related compounds (with similar structures) will typically be present in the matrix. The discriminatory power of such a method can be established by obtaining affirmative results (typically using a reference material of high purity) from samples containing the analyte in a specified matrix, allied with obtaining no response from samples containing no analyte, within the same matrix (for drug substance, the matrix is the entire sample). Additionally, the identification test should be tested against materials that are structurally similar or closely related to the analyte of interest, confirming that in these situations, a positive response is not found. The selection of any potentially interfering compounds should be assessed based on "sound scientific judgment" with respect to the typical interferences that may occur.

With regard to chromatographic methodologies for assay, specificity can be demonstrated by the separation of the main peak from those impurities/degradants or matrix peaks; however, the latter need not be separated from each other. Similarly, a specified impurity assay will need to separate that impurity from the main peak and other impurities/degradants or matrix peaks. In contrast, a method designed to quantify all impurities needs to demonstrate resolution from each other, as well as matrix and main peaks. The necessary resolution of these peaks is dependent on relative size (or concentration) as well as their elution order [25]. Interference from the main peak and other impurities/degradants or matrix peaks is greatest for mutagenic impurities, which often need to be quantified at 1 ppm (parts per million) or less (i.e., 0.0001%), and in these cases even standard ICH impurities present at 0.1% will be 1000× larger than the analyte peak [26]. In those cases, sample cleanup is often required to remove as many interfering peaks as possible prior to chromatographic separation [27, 28].

Specificity is of course predicated on existing knowledge and the method will need to be either revalidated or redeveloped/validated should new (or previously unknown) impurities/degradants be found in the sample. If the synthetic pathway for the drug substance is changed, then the method may not resolve all of the new process impurities and will no longer be fit for purpose. Similarly, a modification to the formulation may result in non-separation of the drug substance or degradant from interfering matrix peaks. Therefore, demonstrating method specificity is often an ongoing and iterative process driven by changes to drug substance or drug product.

Specificity is often demonstrated using spiking experiments, where the pure substance is spiked with impurities and components of the matrix (excipients, other actives, e.g., preservatives, antioxidants, etc.) and appropriate resolution is demonstrated. Alternatively, where reference standards of known impurities or degradation products are not available, specificity can be shown by comparing the test results with another well-characterized orthogonal method, for example, a pharmacopoeial method (for an established product) or a validated but different type of analytical procedure, for example, Nuclear Magnetic Resonance (NMR) (an independent method). Hyphenated techniques, for example, HPLC-NMR, have also been used to assess specificity [29]. Stress testing is often used to demonstrate specificity, but care should be taken not to overstress the drug substance as resolution of secondary or tertiary (or higher) degradation products serves no practical purpose and the typical target degradation value is ca. 10% [30]. It is unlikely that samples from ongoing stability studies (either drug substance or product) will be sufficiently degraded to be suitable for assessing method specificity. For drug substance methods use of "mother liquors" is recommended as this medium can concentrate impurities that are typical not seen under normal circumstances. In addition, the "peak purity" of the main peak also needs to be assessed. This is accomplished by taking spectral slices across the main peak and superimposing these on a reference standard using a diode array detector. Other more specific strategies such as orthogonal projection

approach (OPA) [31], principal component analysis (PCA) [32], etc., are also available. Other approaches such as HPLC-MS (HPLC-Mass Spectroscopy) [33] or re-chromatography using a second system [9] can also be used.

Demonstrating the stability-indicating nature of the method is a critical aspect of stability testing of drug substances and products. Therefore a clear goal of any method development/validation strategy is to avoid mass imbalance that may be caused by insufficient retention on the stationary phase or inadequate resolution of the critical impurities [34]. However, practical experience highlights the fact that despite having a fully validated methods according to ICH Q2(R1) [1], mass imbalance is still often encountered during long-term stability studies [35]. Several studies have demonstrated the importance that matrix aging can have on recoveries in methods, such as disintegration [36], dissolution [37, 38], and HPLC assay and related HPLC impurity assay [39].

Computer simulations can be utilized to optimize analytical throughput as well as specificity [40]. DryLab® can be utilized to optimize separations by modeling the effect of mobile phase compositions, including gradient time and shape, ternary eluent, pH, ionic strength, and temperature [40, 41].

5.2.2 Accuracy

It should go without saying that accuracy needs to be demonstrated across the entire range of the assay. For drug substance/drug product, there are several approaches to establishing method accuracy:

1) The use of an analyte of high purity, for example, reference standard.
2) The use of an "independent procedure" to generate comparable results.
3) The use of other validation parameters, for example, precision, linearity, and specificity, may be used to infer accuracy.
4) Spiking known quantities of drug substance into the formulation matrix.
5) In those cases where components of the formulation matrix cannot be procured, it may be appropriate to spike known quantities of drug substance to the existing drug product.

For impurity methods, accuracy should be determined using the following approaches: (i) spiking known quantities of impurities into the pure drug substance or spiking known quantities of degradants into the pure drug substance and the formulation matrix and (ii) the use of an "independent procedure" to generate comparable results. In all cases, it must be evident to the user how the individual or total impurities are to be reported (see ICH Q6A [6]/Q6B [7]), as either area percent (when the drug substance Rf is used) or percent w/w (when the individual Rfs of the individual impurities/degradants are used).

USP <1226> [4] usefully represents a measured test result as

$$\text{Measured test result} = \text{true value}\left(\text{accuracy}\right) + \text{systematic bias} + \text{random error} \tag{5.1}$$

where both the true value and the systematic bias are constants and the random error is normally distributed with a mean of zero.

ICH Q2(R1) [1] provides guidance on the recommended data set that is required to demonstrate accuracy. Other approaches can be used but will require justification. ICH Q2(R1) [1] requires that nine independent assays should be performed that bracket the specified range and that should include (as a minimum) top, middle, and bottom concentrations of the specified range, for example, three concentrations/three independent replicates. The accuracy data should be recorded as percent recovery of the spiked concentration or as the difference between the mean value and the accepted "true" value, together with the calculated confidence intervals (CIs). No guidance is provided as to what are acceptable recoveries in the case of assay or impurity/degradant assays, or indeed other types of assay. However, the assay recoveries should be typically 100% (±CIs), and greater flexibility is allowable for impurity/degradant assay recoveries (i.e., 80–120% is not uncommon), that is, some level of low or high recovery, that is, bias, may be acceptable in this latter case. In addition, USP <1226> [4] recommends defining three true values across the range, with one value being at the expected value, that is, label claim of the sample. However, if the range has not been fully defined before validation, then the recommendation is to use more than three concentration levels. Hofer et al. [42] modeled the ability of an HPLC assay to rapidly identify large changes (≥0.5%) in the true mean of an assay determination. They modeled the scenario where for the first 50 batches of a new drug substance, the true mean was 99.5% and the standard deviation of the HPLC assay was 0.5%; thereafter the true mean dropped to 99.0%, with the same standard deviation, that is, a 0.5% absolute change. The simulation demonstrated that it is virtually impossible to detect the 0.5% change by trending the HPLC assay data, and more worryingly, if the reviewer does indeed believe that a major change in the process has occurred, it is very difficult to decide when this change occurred and the lack of this vital information will hinder any future investigations into the root cause of the issue. The authors advocated the use of a mass balance approach (100% – % total impurities), where total impurities include related substances, solvents, water, nonvolatile residues, residual metals, etc. They remodeled the previous simulation using a mass balance approach and demonstrated unequivocally that it was straightforward to detect both the change and when the change occurred. Another advantage of this approach is addressing shifts in HPLC assay that occur when reference standards are changed or there is a redesignation of the purity of the existing reference standard. In contrast, EMA [43] reiterated that, when analyzing the active pharmaceutical ingredient (API) and drug product, "whenever possible, an external standard should be used"; in contrast, area normalization (instead of external standard) should be reserved for certain APIs consisting of a "family of compounds."

Incomplete recovery of drug or specified impurities caused by matrix aging has been sparsely reported in the literature. 4-Chloroaniline (PCA) is a known

mutagenic impurity and degradant of proguanil hydrochloride [44] and chlorhexidine [45, 46]. Estradiol is a model steroid that is known to be difficult to recover from matrices in low dose solid oral formulations [47]. PCA was spiked into several solid oral or suspension formulations at levels between 0.5 and 1 μg, reflecting likely ICH M7 [48] control levels (see ICH M7). Initial recoveries in all cases were good (99.9–100.9%; 8.7–10.9% RSD), and the methods had adequate detection limits (DLs) (0.003–0.006 μg/ml) and quantitation limits (QLs) (0.01–0.02 μg/ml). Similarly, estradiol was spiked into various formulations at levels between 9 and 30 μg. Initial recoveries in all cases were good (99.2–100.6%; 0.7–2.0% RSD), and the methods had adequate DLs (0.05–0.08 μg/ml) and QLs (0.18–0.24 μg/ml). In comparison with unstressed samples, large changes in recoveries were seen for both PCA and estradiol in two-thirds of the formulations investigated [39]. PCA recoveries in Paludrine® decreased from 100.2 to 93.1% after 6-month storage at 25°C/60%RH and to 87.6% after 1-month storage at 60°C. PCA recoveries from Diazepam STADA® tablets decreased from 100.9 to 91.7% after 1 month at 40°C/75%RH and in Maaloxan® suspension from 100.6 to 89.6% after 7 days at 60°C. Similarly, recoveries of estradiol formulations were decreased from aged matrices.

For biological molecules, different criteria for accuracy are required. To assess the accuracy of the SE-UPLC (Size Exclusion-Ultra Performance Liquid Chromatography) method for Transferon, the authors calculated the variations in peak areas between batches and between three highly characterized standard "lectures." The method accuracy was then defined as "variations of areas between batches, lectures are less than 3.0% [23]."

5.2.3 Precision

Assay and impurity assay methods need to be assessed for repeatability and intermediate precision (see Table 5.1). Reproducibility is not required for a marketing authorization application but will be typically performed as part of any inter-laboratory assessment when the method is transferred into production.

5.2.3.1 Repeatability

Repeatability, which was historically termed intra-assay precision (or within assay precision), defines the precision obtained using the same operating conditions over a designated short period of time (typically ≤1 day). ICH Q2(R1) [1] recommends that nine independent assays should be performed that bracket the specified range, which should include (as a minimum) top, middle, and bottom concentrations of the specified range, for example, three concentrations/three independent replicates. Alternatively, six independent replicates may be performed at 100% of the proposed target concentration. Repeatability can also be determined using an analysis of variance (ANOVA) approach that is used to assess the overall variability within the "performed series" [16]. In

addition to method repeatability, it is useful to assess HPLC system repeatability (within injection precision) by performing six replicate injections either at the beginning of the HPLC run or from replicate injections of the standard solution used during the method repeatability investigations [9]. This is typically part of any system suitability test (SST). The maximum permitted repeatability for various different drug substance specification limits is outlined in the various pharmacopoeias. For instance, the Ph. Eur. (European Pharmacopoeia), for a drug substance assay limit of 97.0–103.0%, defines the allowable repeatability as 1.1% for five replicate injections.

Transferon, a typical biological product, shows significant batch-to-batch variability, characterized using a SE-UPLC method [23]. Interestingly, precision was not one of the method validation criteria selected. Ten batches of this product, manufactured using the same process, showed considerable intra-batch variability for the area percent of each of the eight main peaks: P1 (mean 6.05, RSD 29.41%), P2 (mean 22.39, RSD 20.56%), P3 (mean 2.53, RSD 27.07%), P4 (mean 12.6, RSD 18.76%), P5 (mean 28.09, RSD 14.35%), P6 (mean 11.08, RSD 19.58%), P7 (mean 0.77, RSD 28.75%), and P8 (mean 15.46, RSD 23.59%). The authors indicated that this level of inter-batch reproducibility (<30% RSD) is typical of biological products and is accepted by regulatory agencies across the world. They cited the case of the Ph. Eur. specification for erythropoietin isoform 6, which has a range from 10 to 30% area, showing 50% variation [49]. In biological products these "related compounds" all have some measure of biological activity, and therefore they are often accepted as being an integral part of the product.

5.2.3.2 Intermediate Precision

Intermediate precision, which was historically termed inter-assay precision, defines the precision obtained using the same operating conditions, typically within the same laboratory, over a designated period of time (typically ≥1 day). ICH Q2(R1) [1] does not provide specific guidance for intermediate precision. Rather, the impact of random variables on the intermediate precision of the method should be assessed. Typical variables that should be investigated include days, analysts, equipment, etc. These variables do not need to be assessed individually; rather the use of a design of experiments (DoE) approach is recommended. Intermediate precision can also be determined using an ANOVA approach, which is used to assess the overall variability within the "performed series" and the variability "between series" [16]. It is worth highlighting that the "concept of intermediate precision" should include a wide range of analytical variability, in terms of people and time and potentially equipment, and that intermediate precision derived from ongoing stability studies, which cover several years in duration, is at the upper limits on intermediate precision variability and can be considered as being very close to reproducibility in nature [50]. Both ICH Q2(R1) [1] and USP (United States

Pharmacopeia) <1226> [4] use a combination of accuracy and precision as this helps to define the interrelationship between bias and precision; the degree to which the bias impacts on the "fitness for purpose" is intrinsically linked to its precision. Therefore, a method having low intermediate precision can tolerate greater bias than those methodologies having increased intermediate precision [4].

Intermediate precision is the most appropriate validation parameter for evaluation of process capability (CpK) and should be interrogated when proposing specification limits. Interestingly, the variability associated with the method is often greater than the variability associated with manufacturing, particularly for drug substance manufacture (see ICH Q6A [6]; ICH Q6B [7]). As a rule of thumb, the standard deviation (σ) of the analytical method should be less than or equal to one-sixth of the proposed specification range. For a 6-sigma process, this should be 1/12th of the proposed specification range, that is, 0.7% or less [21].

Dejaegher et al. [21] indicated that one way of decreasing method variability was to increase the sample/standard weights fivefold (from ca. 32 to >160 mg); this then aligns the sample sizes to those typically seen for titrimetric methods where the precision is significantly better (ca. 0.1–0.5% RSD). Thus, the validation data can impact on the analytical procedure, for example, the number of replicate determinations, size of sample/standards, or the calibration mode [9]. Tsang [51] showed that for any proposed assay specification operating at 3σ, that is, process mean $\pm 3\sigma$, a specification of ±2% (4% range) is equivalent to a total variability of 0.67%; thus the method variability needs to be at least half this value, that is, 0.34%. This is obviously extremely challenging in practice.

Building on this initial work, Ermer [52] used a total of 2915 assays (based on 44 different APIs, manufactured by many different large pharmaceutical companies and 156 different stability studies) to determine a typical HPLC assay precision assessment. The cumulative drug substance intermediate precision for HPLC assays was found to be 1.1% [52]. Hofer et al. [42] reported that the mean intermediate precision values for drug substance HPLC assays were between 0.6 and 1.1%, with ranges between 0.2 and 1.7%.

A risk assessment may be performed (see ICH Q9 [53]) to identify method variables (noise and experimental parameters) that have the potential to impact the method performance and to prioritize which of these should undergo experimental evaluation and which should be fixed/controlled in the analytical procedure. Pohl et al. [8] have proposed how analytical method factors can be evaluated, and this process is depicted in Figure 5.1.

Borman et al. [54] describe a risk assessment that was performed for gas chromatography with a flame ionization detection (GC-FID) method. Figure 5.2 shows the fishbone diagram (using mind-mapping software) that was used to brainstorm and categorize all of the method factors.

Such a risk assessment will identify parameters and factors against which to assess the robustness (see Section 5.2.8) and ruggedness of the method. The assessed risks, as well as scientific and practical issues, aid the design of

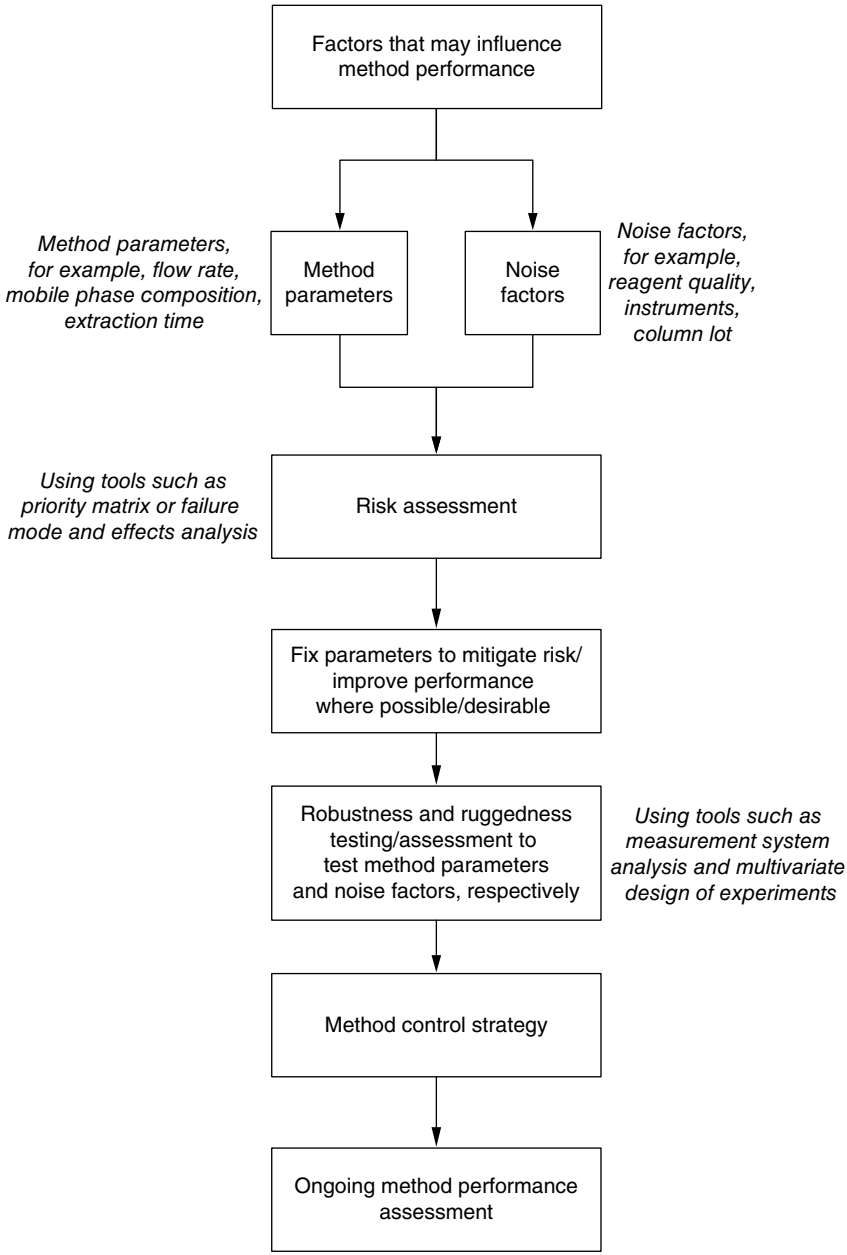

Factors that may influence method performance
Method parameters
Noise factors
Method parameters, for example, flow rate, mobile phase composition, extraction time
Noise factors, for example, reagent quality, instruments, column lot
Risk assessment
Using tools such as priority matrix or failure mode and effects analysis
Fix parameters to mitigate risk/ improve performance where possible/desirable
Robustness and ruggedness testing/assessment to test method parameters and noise factors, respectively
Using tools such as measurement system analysis and multivariate design of experiments
Method control strategy
Ongoing method performance assessment

Figure 5.1 Analytical factor evaluation. *Source:* Based on Pohl et al. [8].

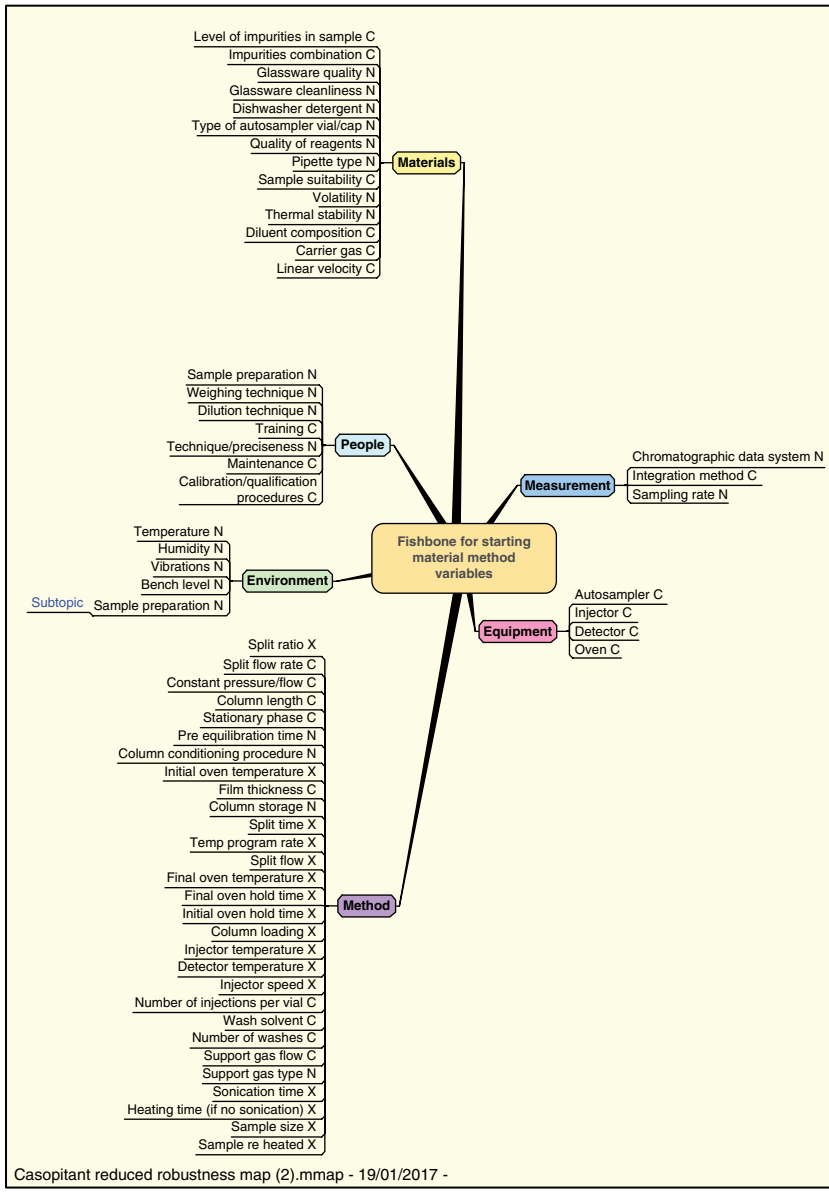


Figure 5.2 Fishbone diagram for GC-FID method factors with enlarged method section. *Source:* Based on Borman et al. [54].

these studies. Ruggedness testing can be defined as the capacity of the analytical method's performance characteristics, over the expected range of analyte, to remain unaffected by variations in any noise factors that the method is likely to encounter under normal conditions [55]. Noise factors are allowed to vary randomly from a specified population and typically cannot be controlled, for example, column batch, trained analyst, and site of analysis. This use of the term encompasses repeatability (same analyst/equipment over a short period of time), intermediate precision, and reproducibility (between laboratories).

The application of a ruggedness investigation provides a more rigorous approach for assessing analytical method precision than the intermediate precision approach articulated in ICH Q2(R1) [1]. Noise factors that are not appropriately controlled should be assessed.

5.2.3.3 Reproducibility

Finally, reproducibility (or between laboratory precision) should be assessed where the analytical procedure needs to be transferred to another site as part of method transfer, in-sourcing or outsourcing. Reproducibility is verified using inter-laboratory trial(s). Ermer [9] indicated that "the analytical procedure should be independent of the actual equipment used." However, it is worrying that reproducibility is often the first time that the method is challenged with equipment that utilize intrinsically different operating procedures, for example, HPLC injectors using fixed versus variable volume loops. It is also worth highlighting that the majority of statistical tests employed during method validation are predicated on the assumption that the experimental data is "only influenced by random variability (i.e., that they are normally distributed)." Data that do not conform to these expectations, often termed "gross errors"—that is, differences of equipment, weighing or dilution procedures, etc.—will impact the data, potentially biasing the outcome [9]. Unfortunately, it is often the case that different laboratories (both within the same organization and outwith that organization) have a different analytical ethos or train their analysts differently and will tend to produce the type of "gross errors" highlighted previously. Skrdla et al. [56] indicated that the "analyst error during weighing/sample transfer and not the target mass (alone) is the larger source of error."

In all cases, the standard deviation (σ), RSD (or coefficient of variation (CoV)), and CI should be reported for each type of precision assessment investigated. Interestingly, the ICH impurity guidelines for drug substance (ICH Q3A [57]) and drug product (ICH Q3B [58]) state that "below 1.0% the results should be reported to two decimal places; at and above 1.0% the results should be reported to one decimal place… the use of two decimal places for thresholds does not necessarily indicate the precision of the analytical procedure used for routine quality control purposes." Agut et al. [50] reflected that "should not the

rounding of any analytical data reflect its uncertainty?" The authors felt that the ICH statement was intended as a "practical compromise" but that the relationship between the performance of the analytical procedure and the rounding of the reportable data needs to be scientifically consistent. The authors [50] evaluated intermediate precision data gathered from 22 impurities of both drug substance and drug product covering the range of 0.18–0.76% (3.06–11.94% RSD) for drug substance (10 impurities) and of 0.063–0.76% (3.85–12.42% RSD) for drug product (12 impurities). They demonstrated no clear correlation between the precision and the percentage impurity level, confirming that very good precision can be obtained for impurities in the range 0.05–2.00% using modern analytical systems. Reassuringly, they found that rounding to two decimal places as recommended by the ICH guidance is statistically sound.

It is often assumed that the precision of low dose assay methods and impurity methods, particularly mutagenic impurities, will be significantly higher than is typically encountered. However, while true to some extent, it is also true that many of these methods can exhibit surprisingly low precision values (still high in absolute terms). Schulz et al. [39] reported recovery and precision data for PCA (a mutagenic impurity) and estradiol (a low dose steroid). PCA was spiked into several solid oral or suspension formulations at levels between 0.5 and 1 μg. Precision, in all cases, was observed to be acceptable (8.7–10.9% RSD). Similarly, estradiol was spiked into various formulations at levels between 9 and 30 μg, and precision, in all cases, was observed to be good (0.7–2.0% RSD). Jacq et al. [59] demonstrated RSDs of less than 1% for a method for the analysis of sulfonate esters; the level of precision observed was attributed to the effective automation of the entire analysis, including sample preparation.

5.2.4 Linearity

A linear (or direct) relationship should be demonstrated across the entire range of the analytical method. It should be established directly on the API (by dilution of a solution of known concentration) and/or individual aliquots of spiked mixtures of the drug product components using the proposed approach.

Linearity should also be assessed by visual assessment of a graphical representation of analytical responses as a function of the analyte concentration or theoretical content. The linear relationship (if demonstrated) should be assessed by relevant statistical methodology, for instance, by determination of a regression line using least squares methodology. In certain instances, linearity may only be shown between the assay response and the sample concentrations using mathematical transformation of the test data prior to any regression analysis.

The correlation coefficient (R^2), y-intercept, slope of the resultant regression line, and residual sum of squares together with a graphical representation should be determined and reported in tabular format. Additionally, an

assessment of any nonconformity of the actual data points from the determined regression line is useful in demonstrating a linear relationship. However, linearity should not be assumed nor assessed solely on the correlation coefficient, as is unfortunately sometimes the case [52, 60, 61]. The best practice is by visual assessment or plotting residuals, that is, the absolute differences of the experimentally derived data points from the regression plot [60, 62, 63]. In the case of a linear relationship, the residuals should be randomly distributed and show no bias [64].

Certain analytical methods, for example, thin-layer chromatography (TLC) and immunoassays, do not show classical linearity, even after mathematical treatment. In such case, the analytical response should be assessed using an appropriate function of the concentration of the analyte within the sample.

Linearity (if demonstrated) requires five or more concentrations, for example, 0, 25, 50, 75, 100, and 125% of theory [1]. Other approaches, if appropriate, should be described and justified.

5.2.5 Range

The method's range is typically derived from the linearity evaluations and is critically dependent on the intended application of the procedure. It is confirmed by ensuring that the analytical method affords an appropriate level of accuracy, precision, and linearity for the analyte at the extreme edges of its specified range. ICH Q2(R1) [1] specifies that the following ranges should be evaluated:

- For assay of a drug substance or a drug product, the specified range should be 80–120% of the nominal concentration.
- For content uniformity, the specified range should be 70–130% of the nominal concentration [65], unless the type of the dosage form, for example, metered-dose inhalers, warrants a wider range.
- For dissolution testing of modified release products, ±20% over the specified range, unless data from clinical or bio-batches, justifies wider ranges (see Scale-Up and Postapproval Changes—Modified Release (SUPAC-MR [66]) guidance). Thus, if the specification for a modified release product has three time points (1 h, mean 20%; released; 6 h, mean 50%; and 12 h, NLT 85%) released, then the range would be 0–110% released of the nominal label claim.
- For the assay of a specified impurity, the specified range should be from the reporting level of the impurity to 120% of the specification limit, that is, for a limit of 0.2% then range is 0.05–0.24%.
- For the assay of a specified mutagenic impurity, the DL/QL should be aligned with the level at which that impurity must be controlled, that is, the staged threshold of toxicological concern (TTC) (now term less than lifetime (LTL) exposure; see ICH M7 [48]), and the specified range covers the validated range up to the staged TTC limit or it just covers the staged TTC (for limit tests).

- If both drug assay and impurity assay are performed together as a combined test (typically not recommended) and only a single standard is used representing the nominal concentration, that is, 100%, then the specified range should cover the reporting level of the impurities, that is, 0.05–120% of the assay specification. In this case, it would be best to also use a 0.05% standard as well; otherwise the single standard would need to cover a dynamic range in excess of 10^3, and the precision of the impurity data could be expected to be compromised.

5.2.6 Detection Limit (DL)

The DL (sometimes termed limit of detection (LOD)) and the QL (sometimes termed limit of quantitation (LOQ)) are two related quantities. However, only one is required for any particular use, that is, QL for quantitative tests and DL for qualitative tests [4]. There are several strategies available for determining the DL of the method, which are dependent on whether the methodology is instrumental or noninstrumental in nature [1]. In addition, other approaches may be acceptable, with appropriate justification.

5.2.6.1 Visual Evaluation

This approach may be utilized for both noninstrumental and instrumental analytical procedures. The DL is assessed by analyzing samples with known concentrations of analyte and by extrapolating the minimum level at which the analyte can be dependably assessed. For titrations, where the endpoint is often determined by observing a color change using visual assessment, this may necessitate some corroboration from another analyst, or the colorimetric endpoint may require appropriate lighting or a complementary background, for example, white background.

5.2.6.2 Signal to Noise

This approach can only be utilized for analytical methods exhibiting some level of baseline noise, for example, chromatographic separation procedures. The signal-to-noise (S/N) ratio is determined by comparing signals from samples with known low levels of the analyte and those from blank sample similarly prepared, thereby allowing the determination of the lowest concentration at which the analyte can be dependably detected. S/N ratios of about 3:1 are generally acceptable for determining the DL [1].

5.2.6.3 Standard Deviation of the Response and the Slope

The DL can also be expressed as $3.3\sigma/S$, where σ is the standard deviation of the analyte response and S is the slope of the calibration line. Pragmatically, laboratories will often select the maximal "worst-case value" for the standard deviation. The standard deviation (σ) may be estimated using several different approaches [1].

5.2.7 Quantitation Limit (QL)

The same strategies are applicable for determining the QL of the method. The QL is required for quantitative tests, irrespective of the form of the specification's acceptance criterion [1]. Thus, for example, for a limit test, the QL needs to be lower than the stated limit, that is, NMT 0. XX%, such that the test may substantiate whether the amount of analyte passes or fails the allowable specification limit [4].

5.2.7.1 Visual Evaluation

The QL is assessed by analyzing samples with known concentrations of analyte and by extrapolating the minimum level at which the analyte can be dependably quantified [1].

5.2.7.2 Signal to Noise

S/N ratios of 10 : 1 are generally acceptable for determining the QL [1].

5.2.7.3 Standard Deviation of the Response and the Slope

The QL can also be expressed as $10\sigma/S$, where σ is the standard deviation of the analyte response and S is the slope of the calibration line. The standard deviation (σ) may be estimated using several different approaches, for example, standard deviation of the blank and calibration curves [1].

5.2.8 Robustness

Method robustness is dependent on the type of procedure being assessed. Robustness testing evaluates the influences of deliberate variations in procedure-related method parameters [1]. (The procedure-related method parameters encompass the entire procedure related to the analytical measurement, e.g., sample preparation, and not merely the instrumental parameters.)

Ideally, method robustness should be assessed during the method development phase [67–69] and critically challenged during the method transfer phase. However, this contrasts with the historical reality. Typically, robustness testing is performed as the final step in the validation process, often leading to unexpected findings during method transfer [40, 70, 71].

As mentioned in Section 5.2.3, a risk assessment can be used to identify method parameters that should be assessed using a robustness study. Having identified the parameters to be assessed for robustness, the ranges to be evaluated are then chosen. For those "continuous" method parameters (e.g., temperature, pH, and initial percent organic component for HPLC gradient methods), the range will usually be chosen to encompass, but not greatly exceed, the likely variation in typical use at specified nominal conditions. This contrasts with some situations for processes where the aim is to develop a much wider design space (see ICH Q8(R2) [2]) within which the process set point can then be moved. For categorical method parameters, the categories

will be chosen to encompass those likely to occur in routine use (e.g., manual method of shaking) or where some flexibility may be required (e.g., selection of HPLC column type).

Certain method parameters may be susceptible to variability (critical parameters), and those operating conditions should be appropriately controlled, or a cautionary statement(s) should be added to the procedure. One obvious consequence of any robustness assessment is that a series of SSTs (see Section 5.2.9) are established to ensure that the legitimacy of the analytical procedure is maintained whenever the method is used. Indeed, if the SSTs are not met, then the method cannot be used to generate GMP (Good Manufacturing Practice)-compliant data.

Examples of some typical variations are:

- The stability of the analytical solutions
- The stability of the analyte during the analytical procedure
- The optimal extraction time

For HPLC procedures, examples of typical variations are:

- The influence of slight changes of pH in a mobile phase (especially if the analyte is ionizable and these changes can impact on the ratio of ionized/unionized analyte in solution).
- The influence of slight variations in mobile phase composition (especially the impact on any critical separations or elution orders).
- The influence on slight variations in buffer ionic strength (especially the impact on any critical separations or elution orders).
- The influence of different columns, that is, different lots and/or suppliers; subtle changes in carbon loading, porosity, etc., (especially the impact on any critical separations or elution orders).
- The influence of temperature (especially the impact on any critical separations or elution order) and to assess whether the method needs to have the temperature accurately maintained using a column oven. The latter variable may be critical for transfer of methods to equatorial countries (climatic zones IIIA, IVa, and IVB).
- The influence of flow rate (especially the impact on any critical separations or elution order).
- The influence of solvent mixing in the preparation of mobile phase mixtures for gradient analysis.
- The influence of injection volume, that is, fixed loop versus variable loop injectors (especially the impact on accuracy and precision).
- The influence of detection wavelength, that is, bandwidth, peak width, threshold, etc. [72].

Robustness can be evaluated using a one factor at a time (OFAT) or a DoE approach. The OFAT approach varies one factor and then compares the output with that of

an experiment with all factors at nominal values. The most significant deficiency of this approach is that when factors are assessed using incremental modifications, the effects are assessed for a "smaller domain around the nominal levels" compared with the DoE approach [40]. Using a DoE approach, any factor effect is averaged across the entire domain, and it is a more realistic reflection of typical occurrences. The univariate OFAT approach takes longer and requires more experiments, and the importance of individual factor interactions is difficult to accurately assess [67]. The experimental design approach is clearly preferential and is equivalent to finding the design space for a method (see ICH Q8(R2) [2]). The design space of an analytical method has been termed the "method operable design region" (MODR) [73]. Kormány et al. [40] performed Plackett–Burman and full, fractional, and asymmetrical factorial designs, allowing the investigation of 3–15 HPLC variables based on 8–16 experiments. In addition to "continuous quantitative factors," for example, mobile phase composition, gradient program, flow rate, pH, temperature, etc., qualitative factors, such as stationary phase, or indeed the laboratory, can be assessed via the factorial design [74]. However, because robustness testing is typically a long and exhaustive process, certain groups have looked at the opportunities offered by commercial software, for example, DryLab, to speed up this process [40]. These authors assessed the reliability of predicted retention times and resolution factors compared with experimentally generated data using amlodipine and seven related substances. They demonstrated that optimal theoretical separation could be predicted for all five stationary phases studied within the same preferred time frame (<4 min) by appropriate adjustment of the key variables (gradient conditions, pH, and temperature).

Awotwe-Otoo et al. [75] utilized QbD (see ICH Q8(R2) [2]) approaches to develop a robust reversed-phase HPLC method for the separation of four hydrolytic degradation peptides arising from protamine sulfate. A Plackett–Burman DoE together with multivariate regression and Pareto ranking analysis demonstrated that the injection volume, mobile phase, column temperature, and pH were critical factors ($p < 0.05$) affecting resolution and tailing of the hydrolysis peaks. Then a Box–Behnken DoE with "response surface methodology" was used to interrogate the main interactions. Finally, a desirability function predicted peak resolutions between 1.99 and 3.61 and tailing factors between 1.02 and 1.45 for the four peaks. The optimized method was then validated as per ICH Q2(R1) [1] criteria and demonstrated a DL of 0.015 mg/ml and a QL of 0.06 mg/ml. The method was used to assess different levels of the four peptide hydrolysis products of protamine sulfate from five different sources.

Schmidt and Molnár [76] used QbD to develop and validate an UPLC method for ebastine. They assessed the robustness of the method with respect to six parameters: ternary composition and flow rate of eluent, gradient time, start and end gradient concentration, and temperature. The resultant 729 experiments were performed in silico and used to construct a design space (see ICH

Q8(R2) [2]), where the critical resolution (NLT 2.0) was achieved in all cases and in less than 4.0 min. This allowed a 40-fold increase in sample throughput compared with the existing Ph. Eur. monograph.

5.2.9 System Suitability Test (SST)

During method development and subsequent validation, the critical performance parameters (CPP) of the analytical procedure need to be identified so that "discriminating SSTs" can be designed [9]. System suitability is often defined using resolution of the two components that elute closest to each other. Failure to achieve this critical resolution demonstrates that the method is not performing optimally and investigations should be performed to address this problem before further use (see Section 5.2.1). However, if the acceptance limits for system suitability are too restrictive, then the method may not be sufficiently robust (fit for purpose), which will become evident during routine use in quality control (QC), if not sooner, that is, during method transfer [9].

The SST is a fundamental part of most analytical procedures (particularly chromatographic separation procedures). The SSTs are predicated on the notion that the entire system consisting of equipment, electronics, analytical operations, and samples to be analyzed constitutes an integrated system that can be assessed as such. The SST parameters are dependent on the type of procedure being validated. The interested reader should consult the various pharmacopoeias for any additional information regarding SSTs [77, 78]. Schmidt and Molnár [76] used the method robustness data to perform a risk assessment (see ICH Q9 [53]) that supported the use of a single SST, that is, the resolution of a critical pair of impurities showing the lowest resolution (impurities C and D), and the resolution was fixed at NLT 2.0.

5.3 Comparison of Regulatory Criteria for Method Validation

The ICH Q2(R1) [1] guidelines have different requirements for different tests, that is, identification, assay, and impurity or related substances tests (see Table 5.2).

Although the ICH Q2(R1) [1] guidelines are the most widely accepted global guidelines to demonstrate "fitness for purpose" in the field of bioanalysis, the regional guidelines from EMA [79] and FDA [80] provide more detailed and stricter acceptance criteria (see Table 5.3). In addition to the standard ICH Q2(R1) [1] validation criteria, additional criteria such as carry-over, matrix effects, and dilution integrity and in-depth assessment of the stability of the analyte under different operating conditions are required. This is because bioanalysis typically uses highly sophisticated hyphenated techniques, for example, HPLC-MS-MS. Both supranational guidances require additional concentration levels and more replicate values than the ICH Q2(R1) [1] guideline.

Table 5.2 ICH Q2(R1) [1] requirements for different methods.

	Type of method			
Validation parameter	**Identity**	**Assay[a]**	**Impurity (limit test)**	**Impurity (assay test)**
Specificity (2)[b]	Yes	Yes	Yes	Yes
Accuracy	No	Yes	No	Yes
Repeatability[c]	No	Yes	No[d]	Yes
Intermediate precision[c]	No	Yes	No	Yes
Reproducibility[c]	No	No[e]	No	No[e]
Linearity	No	Yes	No	Yes
Range	No	Yes	No	Yes
DL	No	No	No	No[f]
QL	No	No	Yes	Yes

[a] Content/potency. For dissolution it is validation of the measurement technique. The apparatus is routinely calibrated (see appropriate pharmacopoeia).
[b] Typically two tests are used. Lack of specificity of one analytical procedure can be addressed by use of other orthogonal analytical procedure(s), for example, HPLC RT and IR could be used.
[c] Repeatability, intermediate precision, and reproducibility are all subdivisions of precision.
[d] Some elements of precision are typically performed to provide assurance of any given limit test.
[e] In cases where reproducibility has been determined, intermediate precision is not required. However, the transferring laboratory in a method transfer exercise will typically still perform intermediate precision, and both laboratories will generate reproducibility data.
[f] May be required and is typically reported along with QL.

5.4 Other Chromatographic Tests

5.4.1 Gas Chromatography (GC)

GC predates HPLC but still continues to enjoy niche applications in modern analytical chemistry, for example, analysis of volatile organic compounds (VOCs) or residual solvents. However, the last 10 years have seen a renascence of this established technique, particularly in its hyphenated format, that is, GC-MS, and its application to the determination of low levels of volatile alkylating agents (mutagenic impurities) for which it is particularly well suited [81]. Recent focus has centered on headspace (HS) or static headspace (SHS) approaches, as this reduces matrix interference, particularly from the API, thereby increasing sensitivity, selectivity, and probably robustness (less carry-over potential). In addition, derivatization within the vial can enhance sensitivity [59], and the use of ionic liquids can raise the activity coefficient of the analyte compared with other

Table 5.3 Global regulatory criteria for method validation.

Validation parameter	ICH Q2 (R1)	EMA guidance	FDA guidance
Selectivity	Yes	Yes (6)	Yes (6) (no interference)
Carryover	No	Yes (<20% LLOQ)	No
DL	Yes	No	No
QL	Yes	LLOQ (±20%; ±20% RSD) ULOQ (±20%; ±20% RSD)	LLOQ (±20%; ±20% RSD) ULOQ (±20%; ±20% RSD)
Linearity	Yes (5)	Yes (6)[a] (±15%; ±20% at LLOQ)	Yes (6–8) (±15%; ±20% at LLOQ)
Range	Yes	Yes (defined by LLOQ and ULOQ)	No
Accuracy (%)	Yes (3/3)	Yes (4/5) (±15%; ±20% at LLOQ)	Yes (3/5) (±15%; ±20% at LLOQ)
Recovery	No	No	Yes (3) (precise and consistent)
Precision	Yes[b] (3/3)[c]	Yes (4/5) (±15%; ±20% at LLOQ)	Yes (3/5) (±15%; ±20% at LLOQ)
Dilution integrity	No	Yes (5) (±15%; ±15% RSD)	No
Matrix effects (%)	No	Yes (6) (≤15% RSD)	No
Reproducibility	No[d]	No[c]	No[d]
Stability	No	Yes[e]	Yes[e]
SST	Yes	No	No

[a] Analyzed in replicate.
[b] Precision split into repeatability (within day) and intermediate precision (between day).
[c] Values in brackets reflect the number of samples and the number of replicates.
[d] Inter-laboratory exercises. Not typically required.
[e] Stability of stock and working standards, stability of the analyte in the sample matrix, and extended long-term registration stability.

interfering components, showing interesting selectivity as well as potential enhancing sensitivity [82]. Other approaches to enhancing selectivity and sensitivity were reported by Snow and Bullock [83]. Cheng et al. [84] recently reported on the development and validation of a generic static HS-GC method for the determination of residual solvents, specifically the 44 class 2 and 3 solvents (see ICH Q3C(R5) [85]), in drug substance. Validation was as per ICH Q2(R1) [1] guidelines. Specificity investigations showed that baseline separation (from each other and from the diluents, dimethyl sulfoxide (DMSO)) was achieved for 75% (33/44) of the solvents tested. The authors

indicated that drug substances having more than five residual solvents at or around the DL were rare (combination of optimization of API crystallization, isolation, and drying stages (see ICH Q8(R2) [2]). In this publication, Cheng et al. [84] indicated that for the four APIs selected for investigation, they had between 2 and 4 solvents present at, or near to, their QLs. On that basis the authors indicated that specificity was appropriate for its intended use. Linearity was assessed over a large dynamic range of 0.2–15,000 ppm using 10 concentrations for each of the residual solvents. The R^2 values were in the range 0.9990–1.0000 for all 44 solvents; the intercepts were less than 2% of the most concentrated standard, that is, 20–900 ppm, indicating that the linear range is broader than recommended in the ICH Q2(R1) [1] guidelines, that is, 50–5000 ppm.

Accuracy was determined in the range 20–900 ppm using duplicate preparations of the 44 solvents. The method bias was less than or equal to 2.69% of the true values and was deemed to be fit for purpose. Accuracy was also determined by spiked recoveries at two different concentrations (20–900 and 2–90 ppm) for four model APIs. Spiked recoveries were typically in the range 70–115%, suggesting that the specific interferences likely to be encountered, that is, volatile impurities and impurities in DMSO, should be surmountable, but the authors did highlight some problematic issues, that is, methanol in DS-1 and DS-4 and 2-methoxyethanol in DS-1 and DS-3, which may require additional method validation, or typical hydrocarbon residual solvents may require smaller API sample size, that is, 20–30 mg.

Both repeatability and intermediate precision were assessed based on six replicates at both the working concentration (20–900 ppm) and a lower concentration (2–90 ppm). For repeatability studies, the precision for all analytes was between 0.57 and 2.28% RSD at the higher concentration and 1.56–9.43% RSD at the lower concentration. Similarly, the intermediate precision for all analytes was between 0.69 and 2.19% RSD at the higher concentration and 1.44–10.15% RSD at the lower concentration. For this HS-GC assay the repeatability and intermediate precision was very similar and was again fit for its intended purpose. The precision data for spiked recoveries from model APIs was not reported.

The DLs and QLs were determined using the S/N ratio approach. The DLs ranged from 0.02 to 7.41 ppm, whereas the QLs ranged from 0.07 to 24.70 ppm. The authors attributed the wide range due to the physicochemical differences between the analytes, for instance, solvents containing chlorine, oxygen, or nitrogen have a lower combustion capacity than hydrocarbons by GC-FID methods, leading to lower sensitivity and correspondingly higher DL/QL values. However, the method is still fit for purpose as the QLs are still lower than the corresponding ICH Q3C(R5) [85] limits for residual solvents (≥50 ppm).

Although toxic solvents, that is, class 1, are typically avoided in a QbD paradigm (see ICH Q8(R2) [2]), some authors have developed methodology for

these class 1 solvents (see ICH Q3C(R5) [85]), that is, benzene, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethane, and 1,1,1-trichloroethane (the latter for environmental considerations). Due to the increased toxicity, enhanced sensitivity is required for these solvents.

Pavón et al. [86] reported on the development and validation of a programmed temperature vaporizer (PTV)-fast GC-MS (single ion monitoring (SIM)) method for class 1 solvents. The method was validated as per most of the requirements of ICH Q2(R1) [1]. Specificity was demonstrated by a mixture of chromatography and SIM (Single Ion Monitoring). 1,1-Dichloroethane was resolved from the other four analytes (Rt 3.69 min); however, 1,2-dichloroethane and 1,1,1-trichloroethane (Rts 5.69 and 5.73 min) were unresolved, as were carbon tetrachloride and benzene (Rts 6.00 and 6.06 min). SIM in the extracted ion chromatogram was evaluated. Linearity was assessed using triplicate injections of a series of standard solutions prepared for each of the five analytes over the concentration ranges evaluated. In all cases, the analytes demonstrated linearity ($R^2 > 0.99$) and the intercepts included zero in all circumstances. Repeatability was performed on five replicates at S/N values of 3, and this was satisfactory (≤12% RSD). No intermediate precision or range validation was reported. The DLs were in the range 4.9–7.9 ppt, and the QLs were assessed using the same approach, that is, $10\sigma/S$, and were in the range 15–24 ppt. This validation data shows that the method is fit for purpose and has the requisite sensitivity to analyze class 1 solvents (2–8 ppm). Note that 1,1,1-trichloroethane has a higher limit of 1500 ppm and was included because of environmental considerations according to ICH Q3C(R5) [85] (see ICH Q3C(R5)). The method compares favorably with other literature methods. Fliszar et al. [87] used the less sensitive HS-GC-FID approach for the determination of benzene and obtained a DL of 100 ppb, with an appropriate precision (2.7% RSD). Pavón et al. [88] used HS-GC-MS, but without the PTV adaptor, and showed slightly higher DLs of 8 and 42 ppb, for benzene and carbon tetrachloride, respectively, and with similar precision (7.2 and 13%, respectively).

For GC procedures, examples of some typical variations that should be studied under robustness are:

- The influence of different columns, that is, different lots and/or suppliers; subtle changes in carbon loading, porosity, etc. (especially the impact on any critical separations or elution orders)
- The influence of temperature (both oven and injection port) or temperature ramping profiles (especially the impact on any critical separations or elution order or stability of analytes)
- The influence of flow rate (especially the impact on any critical separations or elution order)
- The influence of HS extractive procedures

5.5 Non-chromatographic Method Validation

5.5.1 Near-Infrared Spectroscopy (NIR)

NIR has seen extensive use in process analytical technology (PAT) where the key advantages are noninvasiveness and nondestructiveness, fast acquisition times, and minimal sample preparation allowing online or at-line analysis [89]. However, the development, validation, and implementation of quantitative NIR methods are not straightforward as chemometrics is an integral part of data acquisition. As such, several unique challenges are apparent; assembling representative reference libraries and samples and developing and validating predictive models for multivariate data need to be considered [90–92].

A recent critical review of NIR methods [89] highlighted that the following NIR method applications had been validated as per ICHQ2(R1) [1]:

Determination of excipients [93, 94]
Residual water in drug products or manufacturing processes [95, 96]
Drug substance during in-process manufacturing [97, 98]
Drug substance in drug products [93, 95, 96, 99, 100]
Vaccines [101]

From the literature there appears to be three nonstandard (i.e., based on ICHQ2(R1) [1]) approaches to NIR method validation [89].

Firstly, the descriptive approach compares derived statistical parameters (including classical chemometric approaches) with defined acceptance criteria. This appears to be the preferred approach to NIR validation, and the reader is referred to some of the more recent Refs. [102–104]. Some research groups have also used this approach for at least one of the ICH Q2(R1) [1] validation parameters (typically linearity and precision). The linearity approach compares the predicted results from the NIR method with the reference method, typically using the determination coefficient or the correlation coefficient, and values close to 1 are deemed equivalent. However, this approach has been called into question as not being "fit for purpose" to define comparability or exchangeability [105]. The second approach is termed the "difference approach" and uses a hypothesis-based strategy to assess if the observed effects are statistically different from a reference method, using a t-test (or similar), and to assess the accuracy of the methodology (alternatively termed a "trueness assessment"). Unfortunately, many research groups conclude that when the outcome of the hypothesis test is insignificant, then "absence of the effect can be considered true." But "absence of proof of an effect is not proof of absence," [89] and quantitative methods can be deemed valid, that is, absence of bias, whose performances are potentially unacceptable, that is, not "fit for purpose" [89, 105–107]. De Bleye and Chevez [89] observed that there are equivalence approaches that correct this

flawed approach but are rarely used in NIR method validation [106–108]. The final approach is based on tolerance (or prediction) intervals assessing the methods' fitness for use and can be assessed using accuracy profiles, that is, ±5% for assay methods and ±15% for content uniformity methods. De Bleye and Chevez [89] concluded that using standard chemometric figures of merit (RMSE (Root Mean Standard Error)) or the difference approach (comparing relative bias) does not produce methods that are "fit for purpose" with respect to quality, accuracy, or reliability.

5.6 Traditional Method Validation Versus QbD Alternative

Traditional approaches to method validation (ICH Q2(R1) [1]) and transfer represent a one-off evaluation of the method and do not provide a high level of assurance of method reliability. The limited understanding of a method obtained through these traditional approaches has often led to a method passing a technology transfer exercise from development into commercial use, even though critical method variable(s) had not been fully explored, leading to a method failure some months post-transfer. The roots of method transfer failures can usually be traced to insufficient consideration of the routine operating environment during the method validation exercise and lack of an effective process for capturing and transferring the intrinsic knowledge of the development analysts. This issue can be addressed using life cycle approach to method validation and transfer based on three stages: method design, method qualification, and continued method verification.

In this approach method validation is defined as "the collection and evaluation of data and knowledge from the method design stage throughout its lifecycle of use, which establishes scientific evidence that a method is capable of consistently delivering quality data" [108]. The method design stage includes establishing the method performance requirements, developing a method that will meet these requirements, and then performing appropriate studies to understand the critical method variables that need to be controlled to assure the method is robust and rugged.

Having determined a set of operational method controls during the design phase, the next step is to qualify the method to demonstrate that it will operate as intended. Once qualified, any subsequent change to a method or to its operation environment needs to be risk assessed as part of the change control strategy, with appropriate verification studies performed. Where a method experiences a change in operating environment, a method installation exercise will need to be performed. Using an assessment of risk as the basis, appropriate knowledge transfer activities, training activities, and equipment qualification activities can be performed as part of method installation. Once these activities are complete, a method verification exercise may be required. Again, the

degree of verification should be risk based, taking into account the nature of the change, the installation activities, and the potential impact of the change on the method performance characteristics defined in the ATP [8].

The goal of the final stage of the method life cycle, that is, continued method verification, is to continually assure that the method remains in a state of control, that is, fit for purpose. This should include an ongoing program to collect and analyze data that relate to method performance, for example, by setting criteria for replication of samples or standards during the analysis, by trending system suitability data, or by trending the data from the regular analysis of a reference lot. This activity aligns with the guidance in the USP on system performance verification. Close attention should also be given to any out-of-specification (OOS) [109] or out-of-trend (OOT) results generated by the method once it is being operated in its routine environment.

5.7 Future Trends

Although, ICH has discussed implementing analytical QbD approaches, at the time of writing there are no plans to progress with this proposal. Within a QbD approach, predefined objectives should be established in the form of an ATP, which contains the desired method performance requirements for the measurement attribute, for example, dissolution, assay, etc. The ATP defines the appropriate performance of the method and is method independent.

The analytical QbD approach utilizes risk assessments and in-depth systematic evaluations of critical method variables to identify those controls required to ensure that the measurement uncertainty (MU) of a reportable result is controlled to a level that ensures that the method is *fit for purpose*.

Then an MODR is developed describing those specific method parameter ranges that have been evaluated and verified. It is the analytical QbD equivalent of the design space concept (ICH Q8(R2) [2]) that is used during process development. The MODR allows for understanding of a method from a multidimensional perspective and allows change control of method parameters during the life cycle use of the method.

Hopefully, this update will also involve expanding the current remit of ICH Q2(R1) [1] beyond just chromatographic methods, as is stated in the introduction to the current version (ICH Q2(R1) [1]), that is, "Other analytical procedures may be considered in future additions to this document."

5.8 Conclusions

The development and validation of analytical methods that are "fit for purpose" is the fundamental remit of ICH Q2(R1) [1]. By and large this guidance has been very successful in achieving this goal. However, one of the more

common findings in routine GMP and PAI (Pre-Approval Inspection) audits are the non-robustness of analytical methods, leading to OOS or batch failures [110]. EMA recently indicated that "In the context of globalization of manufacturing activities and the developments of new concepts like Quality by Design (QbD), where technology transfer is expected to happen worldwide within and between companies, within the same country, as well as between countries, the need for guidance for transfer of analytical methods has been identified" [110]. However, ICH Q2(R1) [1] and the related pharmacopoeial guidelines for analytical methods are typically used to support a "one-off" validation exercise with little consideration given to verifying how well the method will perform in "real-world" operating conditions [111]. Significant statistically based data are generated, which satisfy the ICH Q2(R1) [1] guidance; however, they are not adequately linked to routine, ongoing method performance. Of the validation criteria cited in ICH Q2(R1) [1], only repeatability, intermediate precision, and reproducibility provide an insight into how the method will fare during routine use, and the latter criteria are only required during method transfer. Indeed, these precision criteria are the only method validation attributes that evaluate the impact of critical analytical factors (CAFs) on the method requirements, which are termed the ATP [111]. Analytical QbD will address a lot of these concerns. In parallel, the pharmacopoeias are embracing analytical QbD as part of their modernization strategies, and the ATP concept may help to address on the key deliverable of establishing method interchangeability [112].

In addition, limited focus is given to method capability in the context of the agreed specification. Ideally, the method variability should be less than or equal to 1/12th of the allowable specification range [21]. Thus, for example, for an API assay method, the method variability should be less than or equal to 0.125% RSD as the allowable specification range is typically of the order of 1.5%, that is, 98.0–99.5% (taking into account the allowable impurity levels). This has led to considerable debate as to whether external standard HPLC methods are actually "fit for purpose" in this context as they cannot achieve this level of precision. In contrast, those same methods operating in the area normalized mode are fully capable of delivering the requisite precision [113]. Similar arguments can be made when specifications are tightened without consideration to the underlying method capability. This can result in changing a fully capable 6-sigma method into a method that is no longer fit for purpose. The introduction of MU of a reportable result (which will need to be controlled to a level that ensures that the method is fit for purpose) should address the issue of method specification interconnectivity. Using MU approaches, it should be immediately apparent whether proposed changes to the filed specification impact on the method's ability to deliver robust and reproducible data.

Combining QbD approaches aligned with a clear understanding of the MU of the procedure in question should provide a clear pathway to the successful development of robust "fit-for-purpose" methods in the 21st century.

References

1 ICH Q2(R1). 2005. Validation of analytical procedures: Text and methodology. Current step 4 version, November 2005.
2 ICH Q8(R2). 2009 Pharmaceutical development. Current step 4 version, August 2009.
3 Borman PJ, Chatfield M, Nethercote P, Thompson D, Truman K. 2007. The application of quality by design to analytical methods. *Pharmaceutical Technology*, 31(10), 142–152.
4 USP <1226>. 2016. Verification of compendial procedures. USP 39, 1646-1647, 2016.
5 ICH Q10. 2008. Pharmaceutical quality systems. Current step 4 version, June 4, 2008.
6 ICH Q6A. 1999. Specifications: Test procedures and acceptance criteria for new drug substances and new drug products: Chemical substances. Current step 4 version, October 6, 1999.
7 ICH Q6B. 1999. Specifications: Test procedures and acceptance criteria for biotechnological/biological products. Current step 4 version, March 10, 1999.
8 Pohl M, et al. 2010. Implications and opportunities of applying the principles of QbD to analytical measurements. *Pharmaceutical Technology Europe*, 22(2), 29–36
9 Ermer J. 2001. Validation in pharmaceutical analysis. Part I: An integrated approach. *Journal of Pharmaceutical and Biomedical Analysis*, 24, 755–767.
10 USP <1033>. 2016. Biological assay validation. USP 39. 862-877, 2016.
11 ISO 5725-1. 1994. Accuracy (trueness and precision) of measurement methods and results—Part 1: General principles and definitions. http://www.iso.org/iso/catalogue_detail.htm?csnumber=11833. Accessed on October 9, 2014.
12 FDA. 1987. General principles of validation. US Department of Health and Human Services, Food and Drug Administration, CDER.
13 AOAC Peer Verified Methods Program. 1987. Manual on policies and procedures, Arlington, US.
14 USP <1225>. 2016. Validation of compendial procedures. USP 39. 1640-1645, 2016
15 Thompson M, Ellison SLR, Wood R. 2006. Guidelines for single laboratory. Validation of methods of analysis. *Pure and Applied Chemistry*, 74, 835–855.
16 ISO 5725-2. 1994. Accuracy (trueness and precision) of measurement methods and results—Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method. http://www.iso.org/iso/catalogue_detail.htm?csnumber=11834. Accessed on October 9, 2014.
17 Pasteelnick LA. 1993. Analytical methods validation. In: Nash RA, Berry IR (Eds.), *Pharmaceutical process validation*, 2nd Edition, Marcel Decker Inc., New York, pp. 411–428.

18 ASTM (American Society for Testing Materials). 2014. The intermediate precision of ASTM international test methods. http://www.astm.org/SNEWS/ND_2010/datapoints_nd10.html. Accessed on October 8, 2014.

19 ASTM (American Society for Testing Materials). 2014. What are repeatability and reproducibility? http://www.astm.org/SNEWS/MA_2009/datapoints_ma09.html. Accessed on October 8, 2014.

20 IUPAC. 2001. Selectivity in analytical chemistry, recommendations for its use. http://old.iupac.org/projects/posters01/vessman01.pdf. Accessed on October 9, 2014.

21 Dejaegher B, Jimidar M, De Smet M, Cockaerts P, Smeyers-Verbeke J, Vander Heyden Y. 2006. Improving method capability of a drug substance HPLC assay. *Journal of Pharmaceutical and Biomedical Analysis*, 42, 155–170.

22 Noll B, Roehl I. 2013. Separation of oligonucleotides and related substances. In: Okafo G, Elder DP, Webb M (Eds.), *Analysis of oligonucleotides and related substances*, The ChromSoc Separation Science series, ILM Publications, St. Albans, pp. 101–156.

23 Medina-Rivero E, et al. 2014. Batch-to batch reproducibility of Transferon™. *Journal of Pharmaceutical and Biomedical Analysis*, 88, 289–294.

24 Cueto-Rojas HF, Pérez NO, Pérez-Sánchez G, Ocampo-Juárez I, Medina-Rivero E. 2010. Interferon α 2b quantification in inclusion bodies using reversed phase-ultra performance liquid chromatography (RP-UPLC). *Journal of Chromatography B*, 878, 1019–1023.

25 Riley CM. 1996. Statistical parameters and analytical figures of merit. In: Riley CM, Rosanke TW (Eds.), *Development and validation of analytical methods*, Elsevier, Oxford, pp. 15.

26 Elder DP, Teasdale A, Lipczynski AM. 2008. The control and analysis of alkyl esters of alkyl and aryl sulfonic acids in novel active pharmaceutical ingredients. *Journal of Pharmaceutical and Biomedical Analysis*, 46, 1–8.

27 Baker, A. 2010. Development of a strategy for analysis of genotoxic impurities. In: Teasdale A (Ed.), *Genotoxic impurities: Strategies for identification and control*, John Wiley & Sons, Inc., Hoboken, pp. 281–304.

28 David F, Jacq K, Van Hoenacker G, Sandra P. 2010. Strategic approaches to the chromatographic analysis of genotoxic impurities. In: Teasdale A (Ed.), *Genotoxic impurities: Strategies for identification and control*, John Wiley & Sons, Inc., Hoboken, pp. 305–350.

29 Branch SK. 2005. Guidelines from the international conference on harmonisation (ICH). *Journal of Pharmaceutical and Biomedical Analysis*, 38, 798–805.

30 Baertschi SW, Jansen PJ. 2011. Stress testing: A predictive tool. In: Baertschi SW, Alsante KM, Reed RA (Eds.), *Pharmaceutical stress testing*, 153, Taylor & Francis, New York, pp. 13–49.

31 Cuesta Sánchez F, Toft J, van den Bogaert B, Massart DL. 1996. Orthogonal projection approach applied to peak purity assessment. *Analytical Chemistry*, 68, 79–85.

32 Wilberg K, Andersson M, Hagman A, Jacobsson SP. 2004. Peak purity determination with principal component analysis of high-performance liquid chromatography-diode array detection data. *Journal of Chromatography A*, 1029, 13–20.

33 Bryant DK, Kingswood MD, Belenguer A. 1996. Determination of liquid chromatographic peak purity by electrospray ionization mass spectrometry. *Journal of Chromatography A*, 721, 41–51.

34 Bakshi M, Singh S. 2002. Development of validated stability-indicating assay methods-critical review. *Journal of Pharmaceutical and Biomedical Analysis*, 28, 1011–1040.

35 Körner A. 2002. Uncovering deficiencies in mass balance using HPLC with chemiluminescence nitrogen-specific detection. *LCGC North America*, 20, 364–373.

36 Karehill PG, Nyström C. 1990. Studies on direct compression of tablets. Part XXII Investigation on strength increase upon ageing and bonding mechanisms for some plastically deforming materials. *International Journal of Pharmaceutics*, 64, 27–34.

37 Choy YW, Khan N. 2005. Significance of lipid matrix ageing on *in vitro* release and *in vivo* bioavailability. *International Journal of Pharmaceutics*, 299, 55–64.

38 Gordon MS, Rudraraju VS, Rhie JK, Chowhan ZT. 1993. The effect of aging on the dissolution of wet granulated tablets containing super disintegrants. *International Journal of Pharmaceutics*, 97, 119–131.

39 Schulz K, Oberdieck U, Backensfeld T, Weitschies W. 2013. Changes in recovery due to drug product matrix ageing as a source of mass imbalances. *Journal of Pharmaceutical and Biomedical Analysis*, 74, 117–125.

40 Kormány R, Fekete J, Guillarme D, Fekete S. 2014. Reliability of simulated robustness testing in fast liquid chromatography, using state-of-the-art column technology, instrumentation and modelling software. *Journal of Pharmaceutical and Biomedical Analysis*, 89, 67–75.

41 Kosko RM, McLaughlin K, Koenigbauer MJ, Lunte CE. 2006. Application of a column selection system and DryLab software for high-performance liquid chromatography method development. *Journal of Chromatography A*, 1122, 186–193.

42 Hofer JD, Olsen BA, Rickard EC. 2007. Is HPLC assay for drug substance a useful quality control attribute? *Journal of Pharmaceutical and Biomedical Analysis*, 44, 906–913.

43 EMA. 2012. Guideline for setting specifications for related impurities in antibiotics. EMA/CHMP/CVMP/QWP/199250/2009 corr.

44 Taylor RB, Moody RR, Ochekpe NA, Low AS, Harper MIA. 1990. A chemical stability study of proguanil hydrochloride. *International Journal of Pharmaceutics*, 60, 185–190.

45 Gavlick WK, Davis PK. 1994. Gas chromatographic determination of p-chloroaniline in a chlorhexidine digluconate containing alcohol foam surgical scrub product. *Journal of AOAC International*, 77, 583–586.

46 Below H, Lehan N. 2004. HPLC determination of the antisceptic agent chlorhexidine and its degradation products 4-chloroaniline and 1-chloro-4-nitrobenzene in serum and urine. *Michrochimica Acta*, 146, 129–135.

47 Orr NA, Sallam EA. 1978. Content uniformity of potent drugs in tablets. *Journal of Pharmacy and Pharmacology*, 30, 741–747.

48 ICH M7. 2014. Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. Current step 4 version, June 23, 2014.

49 Burns C, Bristow AF, Buchheit KH, Daas A, Wierer M, Costanzo A. 2015. Establishment of the Ph. Eur. Erythropoietin reference substance batch 1. *Pharmeuropa Bio and Scientific Notes*, 2015, 103–21.

50 Agut C, Segalini A, Bauer M, Boccardi G. 2006. Relationship between HPLC precision and number of significant figures when reporting impurities and when setting specifications. *Journal of Pharmaceutical and Biomedical Analysis*, 41, 442–448.

51 Tsang PKS, Larew JSA, Larew LA, Miyakawa TW, Hofer JD. 1998. Statistical approaches to determine analytical variability and specifications: Application of experimental design and variance component analysis. *Journal of Pharmaceutical and Biomedical Analysis*, 16, 1125–1141.

52 Ermer J. 2005. Performance parameters, calculations and tests. In: Ermer J, Miller JHMcB (Eds.), *Method validation in pharmaceutical analysis: A guide to best practice*, Wiley-VCH GmbH and Co., KGaA, Weinheim, pp. 88, 21–194.

53 ICH Q9. 2005. Quality Risk Management. Current step 4 version, November 9, 2005.

54 Borman P, Chatfield M, Jackson P, Laures A, Okafo G. 2010. Reduced-method robustness testing of analytical methods driven by a risk-based approach. *Pharmaceutical Technology*, 34(4), 72–86.

55 Borman PJ, Chatfield MJ. 2011. Method ruggedness studies incorporating a risk based approach: A tutorial. *Analytica Chimica Acta*, 703, 101–113.

56 Skrdla PJ, et al. 2009. Use of a quality-by-design approach to justify the removal of the HPLC weight % assay from routine API stability testing protocols. *Journal of Pharmaceutical and Biomedical Analysis*, 50, 794–796.

57 ICH Q3A(R2). 2006. Impurities in new drug substances. Current step 4 version, October 25, 2006.

58 ICH Q3B(R2). 2006. Impurities in new drug products. Current step 4 version, June 2, 2006.

59 Jacq K, et al. 2008. Development and validation of an automated static headspace gas chromatography-mass spectrometry (SHS-GC-MS) method for monitoring the formation of ethyl methane sulfonate from ethanol and

methane sulfonic acid. *Journal of Pharmaceutical and Biomedical Analysis*, 48, 1339–1344.

60 AMC Technical Brief No. 3. 2000. Analytical Methods Committee: Is my calibration linear? http://www.rsc.org/pdf/amc/brief3.pdf. Accessed on January 12, 2014.

61 Loco JV, Elskens M, Croux C, Beernaert H. 2002. Linearity of calibration curves: Use and misuse of the correlation coefficient. *Accreditations and Quality Assurance*, 7, 281–285.

62 Ermer J, Miller JHMcB. 2000. *Method validation in pharmaceutical analysis: A guide to best practice*, Wiley-VCH GmbH and Co., KGaA, Weinheim.

63 Einax JW, Reichenbächer M. 2006. Solution to quality assurance challenge 2. *Analytical and Bioanalytical Chemistry*, 384, 14–18.

64 Renger B, Végh Z, Ferenczi-Fodor K. 2011. Validation of thin layer and high performance thin layer chromatographic methods. *Journal of Chromatography A*, 1218, 2712–2721.

65 USP <905>. 2016. Uniformity of dosage units. USP 39. 736-740, 2016.

66 SUPAC MR. 1997. *Modified release solid oral dosage forms: Scale-up and postapproval changes—Chemistry, manufacturing, and controls; in vitro dissolution testing and in vivo bioequivalence documentation*, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Rockville.

67 Dejaegher B, Vander Heyden Y. 2007. Ruggedness and robustness testing. *Journal of Chromatography A*, 1158, 138–157.

68 Vander Heyden Y, Nijhuis A, Smeyers-Verbeke J, Vandeginste BGM, Massart DL. 2001. Guidance for robustness/ruggedness tests in method validation. *Journal of Pharmaceutical and Biomedical Analysis*, 24, 723–753.

69 Dong MW. 2006. *Modern HPLC for practicing scientists*, Wiley-Interscience, Hoboken.

70 Monks K, Molnar I. 2012. Quality by design: Multidimensional exploration of the design space in high performance liquid chromatography method development for better robustness before validation. *Journal of Chromatography A*, 1232, 218–230.

71 Bliesner DM. 2006. *Validating chromatographic methods*, Wiley_Interscience, Hoboken.

72 Vander Heyden Y, Nijhui A, Smeyers-Verbeke A, Vandeginste BGM, Massart DL. 2001. Guidance for robustness/ruggedness tests in method validation. *Journal of Pharmaceutical and Biomedical Analysis*, 24, 723–753.

73 Borman P, Roberts J, Jones C, Hanna-Brown M, Szucs R, Bale S. 2010. The development phase of an LC method using QbD principles. *Separation Science*, 2, 2–8.

74 Hund E, Vander Hayden Y, Haustein M, Massart DL, Smeyers-Verbeke J. 2000. Robustness testing of a reversed-phase high-performance liquid chromatographic assay: Comparison of fractional and asymmetrical factorial designs. *Journal of Chromatography A*, 874, 167–185.

75 Awotwe-Otoo D, et al. 2012. Application of quality by design elements for the development and optimization of an analytical method for protamine sulfate. *Journal of Pharmaceutical and Biomedical Analysis*, 62, 61–67.

76 Schmidt AH, Molnar I. 2013. Using innovative quality-by-design approach for the development of a stability indicating UHPLC method for ebastine in the API and pharmaceutical formulations. *Journal of Pharmaceutical and Biomedical Analysis*, 78–79, 65–74.

77 USP <621>. 2016. Chromatography. USP 39. 459-471, 2016.

78 Ph. Eur. 2.2.46. 2014. Chromatographic separation techniques. http://library.njucm.edu.cn/yaodian/ep/EP5.0/02_methods_of_analysis/2.2.__physical_and_physicochemical_methods/2.2.46.%20Chromatographic%20separation%20techniques.pdf. Accessed on April 28, 2017.

79 EMA. 2011. Guideline on bioanalytical method validation. EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2** Committee for Medicinal Products for Human Use (CHMP), July 21, 2011.

80 FDA. 2001. *Guidance for industry. Bioanalytical method validation*. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), Rockville.

81 Elder DP, Teasdale A, Lipczynski AM. 2008. The control and analysis of alkyl esters of alkyl and benzyl halides and other related reactive organohalides as potential genotoxic impurities in active pharmaceutical ingredients (APIs). *Journal of Pharmaceutical and Biomedical Analysis*, 48, 497–507.

82 Laus G, Andre M, Bentivoglio G, Schottenberger H. 2009. Ionic liquids as superior solvents for headspace gas chromatography of residual solvents with very low vapor pressure, relevant for pharmaceutical final dosage form. *Journal of Chromatography A*, 1216, 6020–6023.

83 Snow NH, Bullock GP. 2010. Novel techniques for enhancing sensitivity in static headspace extraction-gas chromatography. *Journal of Chromatography A*, 1217, 2726–2735.

84 Cheng C, Liu S, Mueller BJ, Yan Z. 2010. A generic static headspace gas chromatography method for determination of residual solvents in drug substance. *Journal of Chromatography A*, 1217, 6413–6421.

85 ICH Q3C(5). 2011. Impurities: Guidelines for residual solvents. Current step 4 version, February 4, 2011.

86 Pavón JLP, Sánchez MdN, Laespada MEF, Pinto CG, Cordero BM. 2007. Analysis of class 1 residual solvents in pharmaceuticals using headspace programmed temperature vaporization-fast gas chromatography-mass spectrometry. *Journal of Chromatography A*, 1141, 123–130.

87 Fliszar K, Wiggins JM, Pignoli CM, Martin GP, Li Z. 2004. Analysis of organic volatile impurities in pharmaceutical excipients by static headspace capillary gas chromatography. *Journal of Chromatography A*, 1027, 83–91.

88 Pavón JLP, Sánchez MdN, Pinto CG, Laespada MEF, Cordero BM. 2006. Use of mass spectrometry methods as a strategy for detection and determination of

residual solvents in pharmaceutical products. *Analytical Chemistry*, 78, 4901–4908.
89 De Bleye C, et al. 2012. Critical review of near-infrared spectroscopic validations in pharmaceutical applications. *Journal of Pharmaceutical and Biomedical Analysis*, 69, 125–132.
90 ASTM (American Society for testing materials). 2005. Standard practices for infrared multivariate quantitative analysis, E 1665-05.
91 Forina M, Lanteri S, Casale M. 2007. Multivariate calibration. *Journal of Chromatography A*, 1158, 61–93.
92 Rajalahti T, Kvalheim OM. 2011. Multivariate data analysis in pharmaceutics: A review. *International Journal of Pharmaceutics*, 417, 280–290.
93 Karande AD, Heng PWS, Liew CV. 2010. In-line quantification of micronized drug and excipients in tablets by near infrared (NIR) spectroscopy: Real time monitoring of tabletting processes. *International Journal of Pharmaceutics*, 396, 63–74.
94 Blanco M, Alcala M, Bautista M. 2008. Pharmaceutical gel analysis by NIR spectroscopy: Determination of the active principle and low concentration of preservatives. *European Journal of Pharmaceutical Sciences*, 33, 409–414.
95 Mantanus J, et al. 2009. Moisture content determination of pharmaceutical pellets by near infrared spectroscopy: Method development and validation. *Analytica Chimica Acta*, 642, 186–192.
96 Peinado A, Hammond J, Scott A. 2011. Development, validation and transfer of a near infrared method to determine in-line the end point of a fluidised drying process for commercial production batches of an approved oral solid dose pharmaceutical product. *Journal of Pharmaceutical and Biomedical Analysis*, 54, 13–20.
97 Blanco M, Peguero A. 2010. Analysis of pharmaceutical by NIR spectroscopy without a reference method. *TRAC-Trends in Analytical Chemistry*, 29, 1127–1136.
98 Mantanus J, et al. 2010. Building the quality into pellet manufacturing environment: Feasibility study and validation of an in-line quantitative near infrared (NIR) method. *Talanta*, 83, 305–311.
99 Blanco M, Alcala M. 2006. Content uniformity and tablet hardness testing of intact pharmaceutical tablets by near infrared spectroscopy: A contribution to process analytical technologies. *Analytica Chimica Acta*, 557, 353–359.
100 Blanco M, Cueva-Mestanza R, Peguero A. 2010. Controlling individual steps in the production process of paracetamol tablets by use of NIR spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis*, 51, 797–804.
101 Lai X, et al. 2007. Determination of aluminium content in aluminium hydroxide formulation by FT-NIR transmittance spectroscopy. *Vaccine*, 25, 8732–8740.
102 Ito M, et al. 2010. Development of a method for non-destructive NIR transmittance spectroscopic analysis of acetaminophen and caffeine

anhydrate in intact bilayer tablets. *Journal of Pharmaceutical and Biomedical Analysis*, 53, 396–402.

103 Li W, Xing L, Fang L, Wang J, Qu H. 2010. Application of near-infrared spectroscopy for rapid analysis of intermediates of Tanreqing injection. *Journal of Pharmaceutical and Biomedical Analysis*, 53, 350–358.

104 Corredor CC, Bu D, Both D. 2011. Comparison of near-infrared and microwave resonance sensors for at-line moisture determination in powders and tablets. *Analytica Chimica Acta*, 696, 84–93.

105 Rozet E, et al. 2007. Analysis of recent pharmaceutical regulatory documents on analytical method validation. *Journal of Chromatography A*, 1158, 111–125.

106 Bouabidi A, et al. 2010. Critical analysis of several analytical method validation strategies in the framework of the fit for purpose concept. *Journal of Chromatography A*, 1217, 3180–3192.

107 Chatfield M, Borman PJ. Acceptance criteria for method equivalency assessments. *Analytical Chemistry*, 81, 9841–9848.

108 Nethercote P, Borman P, Bennett T, Martin G, McGregor P. 2010. QbD for better method validation and transfer. Pharma Manufacturing, April 2010, 37–49.

109 FDA. 2006. *Guidance for industry: Investigating out-of-specification (OOS) results for pharmaceutical production*, US department of health and human services, Food and Drug Administration, CDER, Rockville.

110 EMA. 2013. Concept paper on the revision of chapter 6 of the EU GMP Guide Quality Control, October 12, 2010, EMA/INS/ GMP/632654/2010. http://www.ema.europa.eu/docs/n_GB/document_library/Regulatory_and_procedural_guideline/2010/11/WC500099260.pdf. Accessed on October 1, 2014.

111 Elder DP, Borman P. 2013. Improving analytical method reliability across the entire product lifecycle using QbD approaches. *Pharmaceutical Outsourcing*, 14(4), 14–19.

112 Grosche O. 2014. Analytical QbD and pharmacopoeial monographs: A vision. EDQM: 50 Years of Leadership in the Quality of Medicines. Strasbourg, October 6–8, 2014.

113 Elder DP. 2014. Are HPC-UV methods fit for purpose as true arbiters of quality for APIs? *American Pharmaceutical Review*, 17(4), 68–72.

6

Impurities in New Drug Substances and New Drug Products

ICH Q3A/B: Key Guidelines in the General Impurity Management Process

Andrew Teasdale[1], David Elder[2], James Harvey[3], and Steven Spanhaak[4]

[1] *AstraZeneca, Macclesfield, UK*
[2] *David P Elder Consultancy, Hertford, UK*
[3] *GSK, Ware, UK*
[4] *Janssen Pharmaceutica NV, Beerse, Belgium*

6.1 Introduction

Within the ICH framework, guidelines regulating the management of impurities in the broader sense started more than two decades ago with the conception of ICH Q3A [1] and ICH Q3B [2].[1] These two guidelines are considered key within the overall ICH quality guidelines framework since they introduced the concept of a thresholded approach to determine when to report, identify, and qualify an impurity (or degradant). Qualification is arguably an important but correspondingly challenging process to define. It encompasses deciding whether or not an identified impurity is, from a safety point of view, below a safe limit (which may or may not be the ICH Q3A or Q3B qualification limits) based on either available or obtained data or generically defined safe limits (like the ICH Q3A or Q3B qualification limits).

Over time safety and quality requirements have evolved and become more prescriptive. Starting with ICH Q3C [3], the guidance issued immediately

1 For almost 20 years since the original versions of both guidelines were issued, they remained largely unaltered, despite minor revisions being made to both. ICH Q3A was first revised in 2002 (ICH Q3A (R1)) and again in 2006 (ICH Q3A (R2)). ICH Q3B was first revised in 2003 (ICH Q3B (R1)) and again in 2006 (ICH Q3B (R2)). For the sake of simplicity throughout the remainder of the chapter, these guidance documents will simply be referred to as ICH Q3A and ICH Q3B (without specific reference to revision status, although this infers the latest versions).

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following Q3A and Q3B, limits have been provided for specific impurities like organic solvents and elemental impurities. These limits were based on the so-called permitted daily exposure (PDE) approach introduced in this guideline. Such an approach can however only be applied for those impurities for which compound-specific safety studies have been conducted. The same approach was adopted for the far more recent ICH Q3D [4].

Another area where initial guidance within ICH Q3A and Q3B has been further expanded relates to genotoxic (mutagenic) impurities. Although not defined in terms of genotoxicity, ICH Q3A [1] and Q3B [2] state that unusually toxic substances may warrant lower thresholds than those generically defined in these guidelines. This is further addressed in the ICH M7 guideline [5] that deals with (potentially) mutagenic impurities. This guideline provides a lower generic safety limit for this type of impurity as well as an approach to define specific limits for mutagenic carcinogens based on the so-called TD_{50} values[2] obtained in carcinogenicity studies performed with these compounds [5].

Thus over time the concept of qualification has become more complex and has extended beyond the scope of ICH Q3A and B.

Interestingly reporting and identification thresholds have remained the same as defined decades ago, even with the implementation of ICH M7. The latter indeed specifically states that actual impurities or degradants include those observed in the drug substance (DS) above the ICH Q3A reporting thresholds and that identification of actual impurities is expected when the levels exceed the relevant identification thresholds as outlined by ICH Q3A for impurities and ICH Q3B for degradants. However, also here a refinement was introduced with the finalization of the ICH M7 [5]. A number of potential impurities or degradants have to be investigated regardless whether they have been identified as actual impurities above the relevant ICH Q3A/B thresholds in the DS. This concerns potential impurities arising from the synthesis of the DS including starting materials, reagents, and intermediates in the route of synthesis from the starting material to the DS. Potential degradants in the DS and drug product (DP) that may be reasonably expected to form during long-term storage conditions also need to be assessed. For these potential impurities/degradants, the most important factor in deciding whether or not assessments are required (as per ICH M7[3] and regardless of ICH Q3 and B defined thresholds) is the probability of their presence in DS or DP at significant levels.[4]

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2 TD_{50} is the dose giving a 50% tumor incidence in carcinogenicity studies.

3 That is, in silico assessment of its potential mutagenicity and, if considered a potential mutagen, of actual levels present in DS or DP.

4 That is, actual levels that are above acceptable safety limits (threshold of toxicological concern (TTC) levels) for mutagenic compounds as defined in ICH M7.

Thus not all potential impurities or degradants need be taken into consideration, however, only that subset that is expected to be present at significant levels[5] into the final DS or DP. For potential impurities the risk of carry-over into the DS can be assessed based on process knowledge and purging factors [6]. For potential degradants knowledge of relevant degradation pathways is used to guide decisions on the selection of potential degradation products to be evaluated for mutagenicity [5], for example, from degradation chemistry principles, relevant stress testing studies, and development stability studies. Further details on this specific part of the impurity management process are provided in Chapter 24 on ICH M7.

Important to acknowledge is that for any "unknown" impurity (i.e., those not belonging to the potential impurities or degradants as described previously), it is considered acceptable to apply the original ICH Q3A and B identification thresholds. This leaves a gap for those "unknown" impurities that might constitute a risk of missing a potential mutagenic impurity that is above the TTC and below the identification threshold. However, given the already very conservative approach underlying the TTC levels and the fact that not all mutagenic compounds will actually be human carcinogens (ICH M7 [5]), this slightly increased potential mutagenic risk is considered to be sufficiently low as not to warrant the effort required to evaluate every impurity and potentially reduce them to below TTC levels. It is recognized that this would make drug development almost impractical given the scale of effort it would require.

Although very helpful to guide industry to produce safe and high quality products, ICH Q3A and B and the other related (and referred to) ICH guidelines still leave a number of gaps. Examples include early development compounds as well as molecules (like biologicals) that are considered out of scope for ICHQ3A and Q3B those where exposure is less than lifetime. These have up to now been addressed either on an ad hoc basis by additional regional guidelines in specific areas (e.g., antibiotics) or by industry best practices or internal company procedures.

In this chapter we will provide an overview of the identified gaps as well as proposals for a more general approach.

Finally, the Q3 series of guidelines, and in particular the Q3A and Q3B, can be viewed as the starting point or basis for impurity management. This chapter therefore aims to address the general flow of impurity management, tying in all aspects that have been covered by other ICH guidelines (ICH Q3C—Residual Solvents [3], ICH M7—Mutagenic Impurities [5], and ICH Q3D—Elemental Impurities [4], all discussed in further detail elsewhere in this book). It will also look to examine other aspects not covered by current guidelines (e.g., biologicals, early development thresholds, etc.).

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5 See Note 4.

6.2 Basic Principles

As indicated in Section 6.1, the basis of impurity management hinges on the principal concept of three defined thresholds: the reporting, identification, and qualification thresholds. As such these three thresholds are core to safeguard the safety and quality of DS and DP, defining key quality aspects of drug manufacture such as classification, identification of impurities, and listing of impurities in specifications. The safety aspects are mainly covered by the identification and qualification thresholds. In ICH Q3A and B, qualification is defined as the process of acquiring and evaluating data that establishes the biological safety of an individual impurity/degradant or a given impurity/degradant profile at the level(s) specified. In practice it means that for impurities (i.e., including degradants, leachables, and mutagenic impurities), it has to be decided whether or not they are, from a safety point of view, below the relevant limit (which may or may not be the ICH Q3A or Q3B qualification limit depending on the classification of the impurity), which is considered to be safe. If this is not the case, it may trigger additional purification or other process-related activities, resulting in lower and acceptable levels of the impurity in question.

The actual applied ICH Q3A [1] or B [2] threshold levels can vary based on the amount of DS administered and whether the impurity/degradant arises in the DS or the formulated product. An overview is presented in Table 6.1.

A question often heard is: "what was the scientific basis when these ICH Q3A and B thresholds were originally defined?" Although hard to answer after more than two decades, it appears they were defined based on empirical experience at the time. The value of 0.05% as applied in ICH Q3A [1] most likely reflected the typical limit of quantification possible using of a standard high performance liquid chromatography (HPLC) fitted with a UV detector. The basis of the identification threshold and qualification threshold is less clear.

ICH Q3A [1] guideline states that for maximum daily doses of DS ≤2 g/day, the qualification threshold is 0.15% or 1 mg/day intake (whichever is lower). This infers that a 1 mg/day limit is considered safe for lifelong exposures to nonqualified impurities. For degradants that could even be higher based on the corresponding ICH Q3B [2] qualification threshold. The appropriateness of this 1 mg/day safe limit for lifelong exposure to non-mutagenic impurities will be discussed in detail in a later section of this chapter.

Regarding the reporting and identification thresholds, technical capabilities certainly have increased to enable reporting and identification at significantly lower levels than the current thresholds. However, taking into consideration the caveat made by ICH M7 for (potential) mutagenic impurities/degradants, from a safety and quality perspective, there is no apparent need to modify these, especially since two decades of experience with the current thresholds did not identify any issue.

Table 6.1 Reporting, identification, and qualification thresholds for DS and DP.

	Amount of DS administered per day in milligrams					
Threshold for	**<1**	**1–10**	**>10–100**	**>100–1000**	**>1000–2000**	**>2000**
Reporting						
In DS (ICH Q3A)	0.05%	0.05%	0.05%	0.05%	0.05%	0.03%
In DP (ICH Q3B)	0.1%	0.1%	0.1%	0.1%	0.05%	0.05%
Identification						
In DS (ICH Q3A)	0.10% or 1.0 mg[a]	0.10% or 1.0 mg[a]	0.10% or 1.0 mg[a]	0.10% or 1.0 mg[a]	0.10% or 1.0 mg[a]	0.05%
In DP (ICH Q3B)	1.0% or 5 µg[a]	0.5% or 20 µg[a]	0.2% or 2 mg[a]	0.2% or 2 mg[a]	0.2% or 2 mg[a]	0.1%
Qualification						
In DS (ICH Q3A)	0.15% or 1.0 mg[a]	0.15% or 1.0 mg[a]	0.15% or 1.0 mg[a]	0.15% or 1.0 mg[a]	0.15% or 1.0 mg[a]	0.05%
In DP (ICH Q3B)	1.0% or 50 µg[a]	1.0% or 50 µg[a]	0.5% or 200 µg[a]	0.2% or 3 mg[a]	0.2% or 3 mg[a]	0.15%

Source: ICH Q3A [1] and ICH Q3B [2]. Courtesy of ICH.
[a] Whichever is lower, expressed either as a % of the DS or as total daily intake (TDI) of the impurity/degradant.

The complex relationship between ICH Q3A and ICH M7 is examined in Figure 6.1. The scenarios presented in this figure cover most if not all situations that could be encountered. In Figure 6.1 potential impurities relate to materials known to be associated with the synthesis of the DS.

For true unknown impurities, that is, impurities below the ID threshold in DS/DP or earlier steps (e.g., impurities in the starting material), identification and further assessment is not required unless the relevant ICH Q3A identification threshold is exceeded. Although ICH Q3A/Q3B clearly predate ICH Q9 (Risk Management) [7], this is consistent with the principles of a risk-based approach. Indeed section 3.1 of the ICH Q3A guideline [1] specifies that the focus of any assessment should be centered on actual and potential impurities emphasizing the need to focus on those that might reasonably be expected.

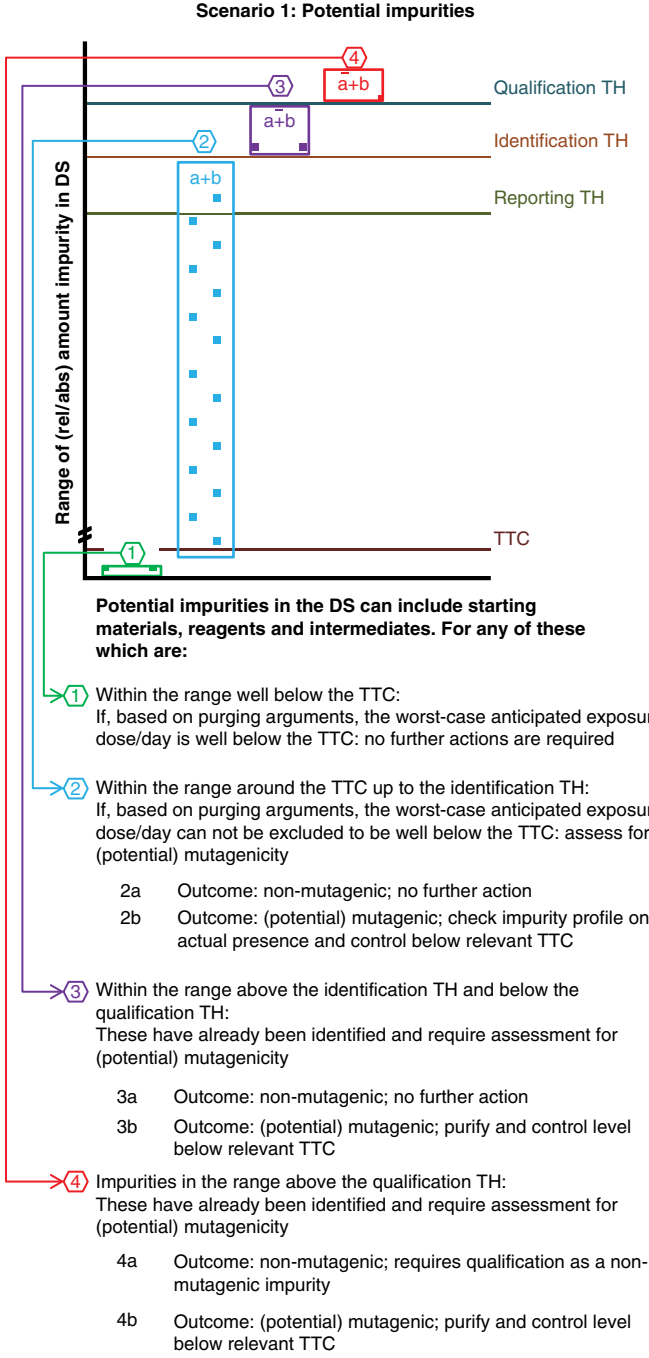
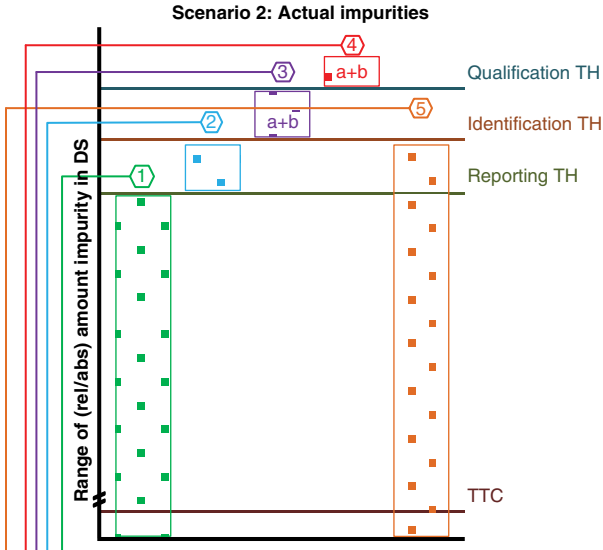

Scenario 1: Potential impurities
Range of (rel/abs) amount impurity in DS
Qualification TH
Identification TH
Reporting TH
TTC
a+b
a+b
a+b
1
2
3
4
Potential impurities in the DS can include starting materials, reagents and intermediates. For any of these which are:
1 Within the range well below the TTC:
If, based on purging arguments, the worst-case anticipated exposure dose/day is well below the TTC: no further actions are required
2 Within the range around the TTC up to the identification TH:
If, based on purging arguments, the worst-case anticipated exposure dose/day can not be excluded to be well below the TTC: assess for (potential) mutagenicity
2a Outcome: non-mutagenic; no further action
2b Outcome: (potential) mutagenic; check impurity profile on actual presence and control below relevant TTC
3 Within the range above the identification TH and below the qualification TH:
These have already been identified and require assessment for (potential) mutagenicity
3a Outcome: non-mutagenic; no further action
3b Outcome: (potential) mutagenic; purify and control level below relevant TTC
4 Impurities in the range above the qualification TH:
These have already been identified and require assessment for (potential) mutagenicity
4a Outcome: non-mutagenic; requires qualification as a non-mutagenic impurity
4b Outcome: (potential) mutagenic; purify and control level below relevant TTC

Figure 6.1 Examples of how ICH Q3A [1] and ICH M7 [5] interact for assessment of impurities in the DS. The examples are divided into two scenarios, one dealing with potential impurities (scenario 1) and one dealing with actual impurities (scenario 2). Indicated on the *y*-axes are the ranges for (relative/absolute) amounts of impurities/degradants in the DS, as well as the various thresholds (TH). The TTC in this context relates to the relevant acceptable intake level as defined in ICH M7 [5], the 1.5 µg/day level being the most conservative one.



Actual impurities or degradants can include those observed in the drug substance above the ICH Q3A reporting thresholds. For any of these which are:

1. Actuals (excluding potential impurities covered under scenario 1) within the range below the reporting TH:
   No action required

2. Actuals (excluding potential impurities covered under scenario 1) within the range above the reporting TH and below the identification TH:
   Only reporting required

3. Actuals (excluding potential impurities covered under scenario 1) within the range above the identification TH and below the qualification TH:
   Reporting, identification and assessment for (potential) mutagenicity required

   - 3a Outcome: non-mutagenic; no further action
   - 3b Outcome: (potential) mutagenic; purify and control level below relevant TTC

4. Actuals (excluding potential impurities covered under scenario 1) within the range above the qualification TH:
   Reporting, identification and assessment for (potential) mutagenicity required

   - 4a Outcome: non-mutagenic; requires qualification as a non-mutagenic impurity
   - 4b Outcome: (potential) mutagenic; purify and control level below relevant TTC

5. Actuals (excluding potential impurities covered under scenario 1) which for any reason have been identified below the actual identification TH should be assessed for mutagenicity

   - 5a Outcome: non-mutagenic; no further action
   - 5b Outcome: (potential) mutagenic; where required purify, and control level below relevant TTC

Figure 6.1 (Continued)

6.3 Reporting and Identification

Reporting and identification are important within the context of impurity classification, qualification, and listing of impurities in specifications. The guideline provides clear guidance in respect to a number of factors that relate to the reporting of data, including number of decimal places, data rounding, and naming/labeling of impurities.

6.3.1 Reporting Requirements

Both guidelines [1, 2] stipulate that analytical results should be provided in marketing applications for all batches of the DS/DP used for clinical, safety, and stability testing, as well as for batches representative of the proposed commercial process. As the guidelines specifically apply to the marketing phase, no definition is provided in terms of requirement during the clinical development phase. However, a similar approach is generally taken in terms of clinical and safety batches.

Both guidelines stress the obvious need to provide quantitative results and these should be presented numerically and not in general terms such as "complies," "meets limit," etc. Again there is little reason to deviate from this approach during the clinical phase.

Both guidelines also state the need to report any impurity at a level greater than (>) the reporting threshold and also to record the total level of impurities observed in any recorded batch of DS/DP. It is also stated that the analytical procedures should be described (see ICH Q2 (R1) [8]).

In terms of number of decimal figures, below 1.0%, the results should be reported to two decimal places (e.g., 0.06%, 0.13%); at and above 1.0%, the results should be reported to one decimal place (e.g., 1.3%). Conventional rules relating to rounding apply. Both guidelines also emphasize the value of reporting results in tabulated format and the need to provide a unique identification for individual impurities, suggesting the use of either a systematic code or based on retention time.

It is highly likely that analytical procedures will change during development (and potentially post-registration) [9]; therefore there is a clear need to ensure that any reported results are linked to the specific analytical procedure used.

6.3.2 Analytical Procedures

Further emphasizing the relationship between ICH Q3A [1]/Q3B [2] and other guidelines, reference is made to the importance of appropriately validated procedures and the need to provide documented evidence of the appropriateness of the test method employed as part of the registration application. Specific requirements are defined in a separate ICH guideline, ICH Q2 (R1) [8]

(see also Chapter 5). The maximum allowable intermediate precision should be stated in the analytical target profile (ATP) [10], this being a measure of "method capability" or "method variability." Typically, method variability should be ≤1/12th of the allowable specification range [11]. It is important to stress the interconnectivity between method variability and specification limits that are subsequently applied (see ICH Q6A [12] and also Chapter 16). If the specification is unrealistically tightened, the method may no longer be fit for purpose at the new limit based on method precision. Another, at times overlooked, requirement described in the guideline is the need to employ a correction factor where levels of an impurity are calculated based on response relative to the active. The requirement to apply such a factor is based on the closeness of the responses of the impurity to that of the active, but no guidance is given as to how close this needs to be before such factors are considered. Generally it is believed that responses outside of the range 0.7–1.3 require application of a response factor. This can have a significant effect especially if levels are underestimated based simply on area. In particular this can have an impact in terms of qualification should the level be revised upward beyond the qualification threshold. This is despite of course the fact that in reality the dose of the impurity remains unchanged. In such instances it may be possible to use existing clinical data as well as preclinical data as part of the qualification of the impurity in question.

6.3.3 Impurity Classification

ICH Q3A [1] in particular classifies impurities in terms of organic impurities/inorganic impurities and residual solvents. Organic impurities are then further subdivided into starting materials/by-products/intermediates/degradation products/reagents, ligands, and catalysts. Solvents and inorganic impurities are of course addressed by their own specific guidance, ICH Q3C [3] and ICH Q3D [4], respectively, these being discussed separately within this overall book within their own individual chapters (see Chapters 7 and 8). Furthermore with the finalization of ICH M7 [5], another important category has been defined, that is, (potentially) mutagenic impurities/degradants that require lower safe levels as those described in ICH Q3A [1] and Q3B [2].

6.3.4 Listing of Impurities in the Specification

The guideline also seeks to provide guidance as to what should be included in terms of impurities on the DS (ICH Q3A [1]) and DP (ICH Q3B [2]) specification. An important aspect of this is the guidance to focus on impurities found in material manufactured using the commercial process. This is an important consideration given that during development it is likely that the synthetic process will have gone through substantive change, both in terms of the synthetic route and process, and it is therefore likely that impurity profiles will have also

changed significantly. Similarly in relation to the formulated product, substantive changes are also likely to have been made to the formulation. Often the final formulation is not defined until phase III clinical trials. Changes to the formulation may also have an impact on the degradation profile observed, certainly in terms of relative levels. In both the case of the DS and the DP, the relevance of impurities should be discussed in the rationale provided in support of the specification.

The section relating to specifications also stipulates acceptance criteria. Again the importance of the relationship between ICH Q3A [1] and ICH Q3B [2] and other guidelines should be emphasized, in this instance the relationship to ICH Q6A—Specifications [12]. Particular reference should be paid to decision trees #1 and #2 within ICH Q6A [12]. These describe the relationship between levels of impurities observed in relevant batches and the level of the impurities qualified in preclinical studies. The primary requirement is that limits should be no higher than those supported by safety data. However, ICH Q3A [1] more contentiously states that levels should be consistent with manufacturing data, that is, levels observed within material produced using the commercial process. Within this subsection it is stated that this can take into consideration variation of both manufacturing process and analytical control methods. Despite this caveat, this "requirement" is often a point of tension, leading to approaches to setting limits based on, for example, mean ±3 SD. It is critical to first understand the intermediate precision [10, 11] of the analytical method before attempting to establish any limits based on process variability as much of the variability may be related to the method rather than the actual process. Failure to do so can lead to a considerable tightening of limits that serves to achieve little in terms of patient safety and yet can lead to substantive numbers of failed batches that severely impact on the viability of the commercial process. Ultimately it is vitally important that a sensible balance between safety and quality factors is achieved when establishing an impurity specification for the active.

6.4 Qualification

Perhaps the most important aspect of the Q3A and Q3B guidelines is the sections relating to qualification. Qualification is defined as being

The process of acquiring and evaluating data that establishes the biological safety of an individual impurity (degradation product) or a given impurity (degradation) profile at the levels specified [1, 2].

The assessment of toxicological qualification of relevant impurities is required prior to initial human studies and is therefore initiated prior to clinical phase I. Typically, impurity levels are assessed based on the impurity profile of the batch(es) used in pivotal GLP 1-month toxicology studies.

Qualification is an iterative process that may require additional studies to be performed should any new impurities and/or degradants be identified at levels above the appropriate qualification threshold in material intended for future clinical studies. Wherever possible a proactive assessment should be made of the likely long-term impurity profile so that material used in preclinical studies is representative of this. Specific consideration should also be given to degradants, in particular the potential levels at the end of the DS/DP shelf life. Where appropriate the deliberate addition of relevant impurities, including degradants, to preclinical material may be considered. Consideration should also be given to the interrelationship between impurities, degradants, and metabolites as the impurity/degradant may already be qualified based on it also being a metabolite.

In both (ICH Q3A and B) guidelines, the process of qualification is represented schematically. Rather than representing these in this chapter, a new revised scheme is presented in Section 6.5.3. This incorporates adjustments based on the more recent guidelines like ICH M7 and proposed approaches for gaps left by the existing guidelines. This is examined through a series of scenarios that outline general approaches applied in order to qualify impurities both during the clinical and approval phase.

Please note that within the following qualification subsections, the reference to impurity indicates non-mutagenic impurities (as opposed to mutagenic impurities as defined by ICH M7).

6.4.1 Use of the Same Batch for Preclinical and Clinical Studies

The simplest approach is to use the same batch for clinical studies as used in toxicity studies even if the structure(s) of the impurity(ies) present have not been fully elucidated they are considered to be qualified.

6.4.2 Different Batches Used in Clinical Studies Compared with That Used for Preclinical Toxicity Studies

In reality it is often not possible to use the same batch of DS for subsequent clinical programs as was used in initial toxicity studies, primarily due to the limited amounts of material. In such a situation impurities can be qualified by comparing the amounts (dose) previously administered in animals with the amount expected to be administered in the clinic. It is important to stress that this should not be based on a simplistic comparison of relative levels present in the respective batches. The comparison is based on absolute dose, not simply concentration. ICH Q3A [1] states, "A level of a qualified impurity higher than that present in a new DS can also be justified based on an analysis of the *actual amount* of impurity administered in previous relevant safety studies." This can be done by calculating the amount of impurity given to animals at the no observed adverse effect level (NOAEL) dose and then comparing this with the

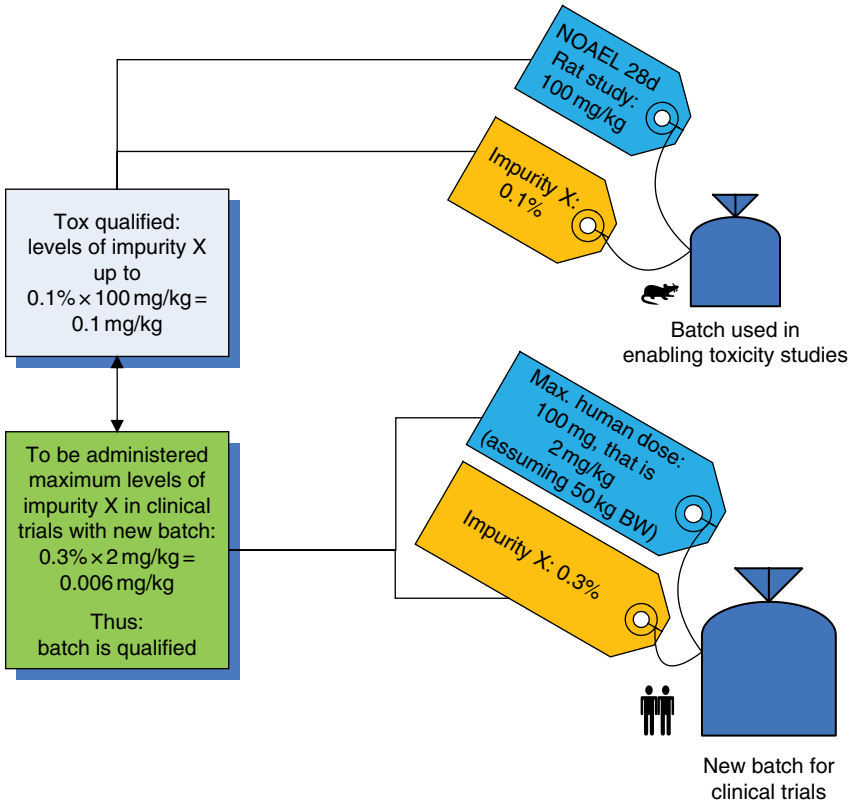


Figure 6.2 Example for qualification of a known impurity in case the clinical batch and the batch for supportive toxicity studies are different.

amount of impurity projected to be given to humans in the proposed clinical studies (in mg/kg/day). Provided the amount given to animals at the NOAEL is greater than that being given to humans, it is considered safe to proceed (see Figure 6.2).

Under certain circumstances it may even be possible to justify higher levels than are provisionally covered. Provided that any toxic effect observed in animal studies is characterized and can be definitively attributed to the parent compound and its pharmacologically, it may be possible to justify the use of higher levels for impurities. It may also be possible to use an effect dose if the observed toxicity is not severe, reversible, and monitorable in the clinic. Such scenarios are typical of areas such as oncology where the pharmacological profile of the active often makes it extremely difficult to identify any specific impurity-related toxicological response.

The two approaches described are those most commonly applied to qualify impurities; however, if these cannot be used, there are a number of alternative approaches.

6.4.3 Justification Based on "Other" Relevant Data

It is often assumed that qualification necessarily involves the conduct of animal studies; however, this is not always required. Before commencing such studies, consideration should be given to preexisting toxicology data, for example, if it is a common reagent used in the chemical synthesis. ICH Q3B [2] states, "Alternatively, adequate data could be available in the scientific literature to qualify an impurity." Precedence of the impurity in another DS or DP with an established safety profile might also provide opportunity to use a first-principles approach to qualification. Additionally compound-specific calculations for acceptable limits can be applied for those impurities that are chemically similar to a known compound class with supporting safety data available, provided that a rationale for chemical similarity can be demonstrated.

6.4.4 Higher Limits Based on Clinical Considerations

Consideration of the clinical indication is another factor for consideration. ICH Q3A [1] states, "Higher or lower thresholds for qualification of impurities can be appropriate for some individual drugs based on scientific rationale and level of concern, including drug class effects and clinical experience. A higher qualification threshold can be appropriate for individual drugs when the level of concern for safety is less than usual based on similar considerations (e.g., patient population, drug class effects, clinical considerations). Proposals for alternative thresholds would be considered on a case-by-case basis." Similarly, ICH S9 [7] states, "It is recognized that impurity standards have been based on a negligible risk, as discussed in ICH Q3A [1] and Q3B [2]. Exceeding the established limits for impurities identified in these ICH guidelines could be appropriate for anticancer pharmaceuticals, or other indications for serious diseases with a short life expectancy, and a justification should be provided in the marketing application. The justification could include the disease being treated and the patient population, the nature of the parent pharmaceutical (pharmacologic properties, genotoxicity and carcinogenic potential, etc.), duration of treatment, and the impact of impurity reduction on manufacturing."

6.4.5 The Impurity Is Also a Metabolite

An impurity may also be metabolite species. Provided that the level produced *in vivo* exceeds the level dosed as an impurity in the DS/DP, the statement in

ICH Q3B [2] applies: "Impurities that are also significant metabolites present in animal and/or human studies are generally considered qualified."

6.4.6 When Levels of an Impurity Cannot Be Qualified by Any of the Aforementioned Approaches

Where the level of an impurity exceeds permissible limits and cannot be qualified on the basis of the principles described, it may not be possible to use it for its intended purpose. In such a situation generally the batch is reprocessed, for example, recrystallization.

Alternatively additional toxicity studies may be performed using either a new batch of drug material with higher levels of the designated impurity or a batch of drug material spiked with the impurity.

However, before initiating such studies, there are other factors (not captured by the ICH Q3A or Q3B guidelines) that can be taken into consideration, that is, especially the duration of exposure.

6.4.7 Alignment to ICH M7: Qualification Testing Strategy

ICH Q3A [1]/Q3B [2] states that qualification in genotoxicity studies be considered. However, it is important to consider this in conjunction with statements made within ICH M7 [5]. Note 1 in ICH M7 states that "ICH M7 recommendations provide a state-of-the-art approach for assessing the potential of impurities to induce point mutations and ensure that such impurities are controlled to safe levels so that below or above the qualification threshold no further qualification for mutagenic potential is required. This includes the initial use of (Q)SAR tools to predict bacterial mutagenicity. In cases where the amount of the impurity exceeds 1 mg daily dose for chronic administration, evaluation of genotoxic potential as recommended in ICH Q3A [1]/Q3B [2] could be considered. In cases where the amount of the impurity is <1 mg, no further genotoxicity testing is required regardless of other qualification thresholds." Realigning this to ICH Q3A/Q3B should therefore negate the need for genetic toxicity testing as part of the qualification of a non-alerting (presumed non-mutagenic) impurity, provided that levels are <1 mg. As stated in ICH M7 [5], for an impurity above this absolute 1 mg level, genotoxicity tests as listed in ICH Q3A [1] can be considered. In this situation and on a case-by-case basis, it has to be decided whether or not the performance of an *in vitro* chromosomal aberration study would add value regarding patient safety. This has to be put in the context that non-mutagenic genotoxicants (chromosomal aberrations) typically have threshold mechanisms and usually do not pose carcinogenic risk in humans at the level ordinarily present as impurities (ICH M7 [5]). Also the performance of a point mutation assay has little effective value since for these impurities the absence of structural alerts for mutagenicity has already been shown.

6.4.8 Is Lifelong 1 mg/Day Exposure to Any (Non-mutagenic) Impurity Safe?

As previously described, ICH Q3A [1] specifies a qualification threshold for synthetic impurities (which are not considered to be unusually toxic, e.g., mutagenic) of 0.15% or 1.0 mg/day intake (whichever is lower) for drugs with a maximum daily dose ≤2 g/day based on lifetime exposure. This implies from a safety perspective that lifetime exposure to 1.0 mg/day of an unqualified non-mutagenic impurity represents a negligible safety risk. As highlighted in the introduction to this chapter, the guideline does not provide any rationale as to the basis for the establishment of this qualification threshold. Is this level scientifically justifiable?

Examining this more closely, a 1 mg/day dose when corrected for human body weight (assumed human body weight of 50 kg) would relate to a NOAEL of 0.02 mg/kg/day (0.02 mg/kg/day × 50 kg human = 1 mg/day) in general toxicity studies. A recent analysis of the databases of subchronic and chronic toxicology studies compiled by Munro et al. [13, 14], Tluczkiewicz et al. [15], by Harvey et al. [16], in conjunction with routine in silico approaches to identify obvious structures associated with unusual toxicity, (e.g. dioxins etc) indicates that the overwhelming majority (> 95%) of chemical substances studied to date have NOAELs that are >0.02 mg/kg/day.

Harvey et al. [16] showed that for the 613 chemical substances examined (industrial chemicals, pharmaceuticals, food substances, and environmental, agricultural, and consumer chemicals), 95% had an NOEL > 0.22 mg/kg/day, that is, providing an 11-fold overage as compared with the required 0.02 mg/kg/day NOAEL to support the 1 mg/day lifelong exposure assumed safe dose.

Harvey et al. [16] also demonstrated that characterization of the compounds in the Munro database in terms of Cramer structural class [17] (I–III: low toxicity, moderately toxic, and contain structural features that permit no strong initial presumptions of safety) does not alter this finding. Analysis specifically of Cramer class III [17] compounds showing the lowest NOAELs, 95% of the compounds still have a NOAEL > 0.15 mg/kg/day. This is 7.5-fold greater than the required 0.02 mg/kg/day NOEL to support the ICH Q3A 1 mg/day lifelong exposure.

Harvey et al. [16] also cited a more recent analysis of the TTC RepDose database ($n = 521$ chemical substances, $n = 397$ of which were not in the Munro database, with no organophosphates or potential genotoxins) [15] this showed that for Cramer class III compounds, 95% had NOELs above 0.038 mg/kg/day. The lower value, in comparison with that defined in the Munro database [14], reflects differences both in chemical space and, more significantly, in the application of allometric scaling to adjust for interspecies differences during standardization of the NOELs. Compared with the NOAEL of 0.02 mg/kg/day required for the lifelong exposure assumed safe dose of 1 mg/day, this still provides an effective twofold overage.

In summary these databases clearly demonstrate that 1.0 mg/day of an unqualified impurity of unknown toxicity represents a negligible risk over a lifetime's exposure.

6.4.9 Duration of Exposure

As highlighted in the introduction, ICH Q3A and ICH Q3B apply specifically to the marketing phase and not during clinical development; the result is that during the development phase, a modified approach, usually company specific, is applied. This generally is based on an empirical modification of the limits defined in the guidelines. In contrast, ICH M7 regarding potentially mutagenic impurities is applicable to all stages of drug development.

ICH Q3A and ICH Q3B are effectively silent as to the impact of duration of exposure. Within the Harvey et al. paper [16], the impact of study duration was closely examined. They focused on thresholded (e.g., non-genotoxic) effects, where toxicities are generally dose and duration dependent, and examined how a cumulative dose approach may be used.

6.4.9.1 Extrapolation to Shorter Treatment Duration Periods: Early Development

Using the 1 mg/day assumed safe dose for lifelong exposure to an unqualified impurity of unknown toxicity as representing a negligible risk of harm, a higher threshold can be derived for shorter-term exposures to an impurity. As described by Gaylor [18], a modification of Haber's law can be used to extrapolate safe levels for short-term durations; this is based on effectively the same principle as used to define the less than lifetime (LTL) dosing (formerly termed the "staged" TTC) for mutagenic impurities [5]. Based on modified Haber's law, absolute impurity levels of 5 mg/day can be considered to be as safe for exposure durations of <6 months as is the 1 mg/day level for lifelong exposure.

Haber's law:

$$c \times t = c' \times t'$$

Haber's law is appropriate for extrapolation to different durations of exposure for conditions where dose rate is not the determining factor and only total dose dictates the biological effect.

It does not sufficiently factor for circumstances where the toxicity hazard might be an acute one, hence the use of the conservative modified form.

Modified Haber's law:

$$c^3 \times t = c'^3 \times t'$$

where

c = safe dose at time t = 1 mg
c' = corresponding safe dose (concentration) at shorter duration t'
t = lifetime—75 years 24,375 days

Therefore, for studies of 6 months' duration or less, $c' = \sqrt[3]{24{,}375/182} = 5$ mg.

For potent drugs that have effective doses in the low μg–mg range, an absolute threshold of 5 mg/day for an impurity is not realistic from either a quality or safety perspective. Therefore, the fivefold higher threshold for the absolute value (1–5 mg/day) is combined with an additional restriction of a relative threshold of 0.7% (i.e., also a fivefold higher relative level as compared with the ICH Q3A [1] qualification level of 0.15%).

In conclusion, a value of 5 mg/day or a relative level of 0.7%, whichever is lower, may be considered to be safe and acceptable for qualification of nonmutagenic impurities during early development clinical trials with durations up to 6 months.

6.5 Extrapolation of Principles to Out-of-Scope Molecules

Another important consideration relates to the types of molecule considered to be within the scope of the guidelines and thereafter the types of impurities associated within them. It is also important to consider how specific types of impurities may ultimately be controlled. ICH Q3A [1] is clear that it applies to synthetic DS, defining other categories such as biological/biotechnological, peptide, oligonucleotide, fermentation product and semisynthetic products derived wherefrom, herbal products, and crude products of animal or plant origin as out of scope. This is reiterated in ICH Q3B [2] in respect to their subsequent formulated products.

One might reflect on this and the significant expansion seen in many of the areas described since the original guidelines were issued. The result is that in many of the areas described, there has been a need to adapt the principles and limits defined in ICH Q3A [1]/Q3B [2] and modify them to apply in the areas concerned.

6.5.1 Antibiotics

One specific area where this has been approached by the European regulators is antibiotics. In 2012 the EMA first published "Guideline on setting specifications for related impurities in antibiotics" [19]. This guideline seeks to provide definitive guidance relating to appropriate reporting, identification, and qualification thresholds for antibiotic treatments. This guideline delineates impurities effectively on the basis of product complexity, differentiating between semisynthetics, fermentation (single product)/fermentation (family), and peptides (specifically antibiotic peptides, e.g., gramicidin) in terms of acceptable limits. The limits concerned are described in Tables 6.2. The values shown relate to the active. Those for semisynthetics are analogous to synthetic DS,

Table 6.2 Thresholds for antibiotics: DS and DP.

Active substances		Semisynthetic	Fermentation (single) (%)	Fermentation (family) (%)	Peptides (%)
Reporting	DS	0.05/0.03[a]	0.10	0.10	0.1
	DP	0.1%	0.15	0.15	0.1
Identification	DS	0.10/0.05	0.15	0.15	0.5
	DP	0.2%	0.2	0.2	0.5
Qualification	DS	0.15/0.05	0.15	0.50[b]/0.2	1.0
	DP	0.2%	0.2	0.50[c]/0.2	1.0

Source: EMA/CHMP/CVMP/199250/2009corr [19].
[a] Lower limit applied where dose >2 g/day.
[b] Structurally closely related impurity.
[c] Structurally closely related impurity.

whereas for fermentation products reporting and identification thresholds are revised upward; however qualification limits, certainly for a single fermentation product, remain the same. This is despite the fact that the guideline makes specific reference to the limited duration of treatment associated with antibiotics. A detailed review of the circumstances surrounding the limits within this specific guideline is out of scope of this review of ICH Q3A [1]/Q3B [2]; however it is nevertheless surprising to see that qualification limits take little account of the limited duration of such treatment and no specific adjustment has been made. Interestingly the qualification threshold for antibiotic peptides is set at 1.0%—no rationale is provided for this adjustment beyond reference to Ph. Eur. General Monograph relating to synthetic peptides [20].

6.5.2 Leachables

For some time, leachables, that is, compounds that migrate into DP or DS from product-contacting materials during manufacturing, storage, or delivery under normal processing and storage conditions, have been recognized as a potential source of impurities. However, leachables have been excluded in the scope of ICH Q3B [2], and there is no specific regulatory guidance. Therefore, during the last decade, the PQRI consortium had published proposed approaches to deal with this issue using very conservative safe levels especially for potential mutagens [21]. With the introduction of ICH M7 at least for (potential) mutagenic impurities, this has been clarified by the statement that the safety risk assessment principles outlined in the ICH M7 guideline can be applied to leachables. Meaning that for leachables, like any other potential mutagenic impurity, the relevant TTC levels as described in ICH M7 apply.

Leachables do not originate from the DS synthesis process and therefore are impurities unrelated to the DS. This is sometimes used as an argument to propose different approaches than those laid down in ICH Q3A or Q3B. However, this principle would apply for at least part of the impurities that are covered under ICH Q3A and Q3B, that is, they have no clear structural relationship with the DS. Moreover, limits as laid down for ICH Q3A, as underpinned in Section 6.4.8, apply to a broad chemical spectrum; therefore it seems acceptable that regarding the thresholded (non-mutagenic) toxicity profile of leachables, similar approaches can be used as laid down in ICH Q3A and Q3B. Or in cases where concerns relate to a leachable for which there is sufficient toxicity data available, a more specific PDE approach as described in ICH Q3C or ICH Q3D can be applied.

6.5.3 Revised Scheme of Impurity Qualification

The scheme presented here as Figure 6.3 is an adaptation of the original ones in ICH Q3A and Q3B regarding the process of qualification incorporating details of ICH M7 and of other sections that may not be based on guidelines.

6.6 Drug Substance Control Strategy Key Points for Consideration

"How pure should a DS be?" is an often asked question for which there is typically no straightforward answer. It is a complex question requiring the consideration and balancing of a number of factors; these include quality, safety, economic, and environmental factors. The following section seeks to examine the various factors and their interrelationship and impact. A simple approach would be to purify the active substance to such an extent that qualification of impurities becomes unnecessary. On the face of it, this would seem ideal especially as this would also seem to lead to an apparent reduction in animal testing and limit human exposure to unknown impurities.

6.6.1 DS Purity During Clinical Development: Impact on Preclinical Testing

"How pure should my DS be at a given stage in development?" is a further refinement of this question. While it may seem sensible to set a target in terms of quality, it is impractical at the beginning of the development process to categorically define the ultimately intended impurity profile based on lack of available information. This can only be achieved once the route and process have been established and a robust understanding of the process has been achieved. The qualification of impurities is a parallel activity to this.

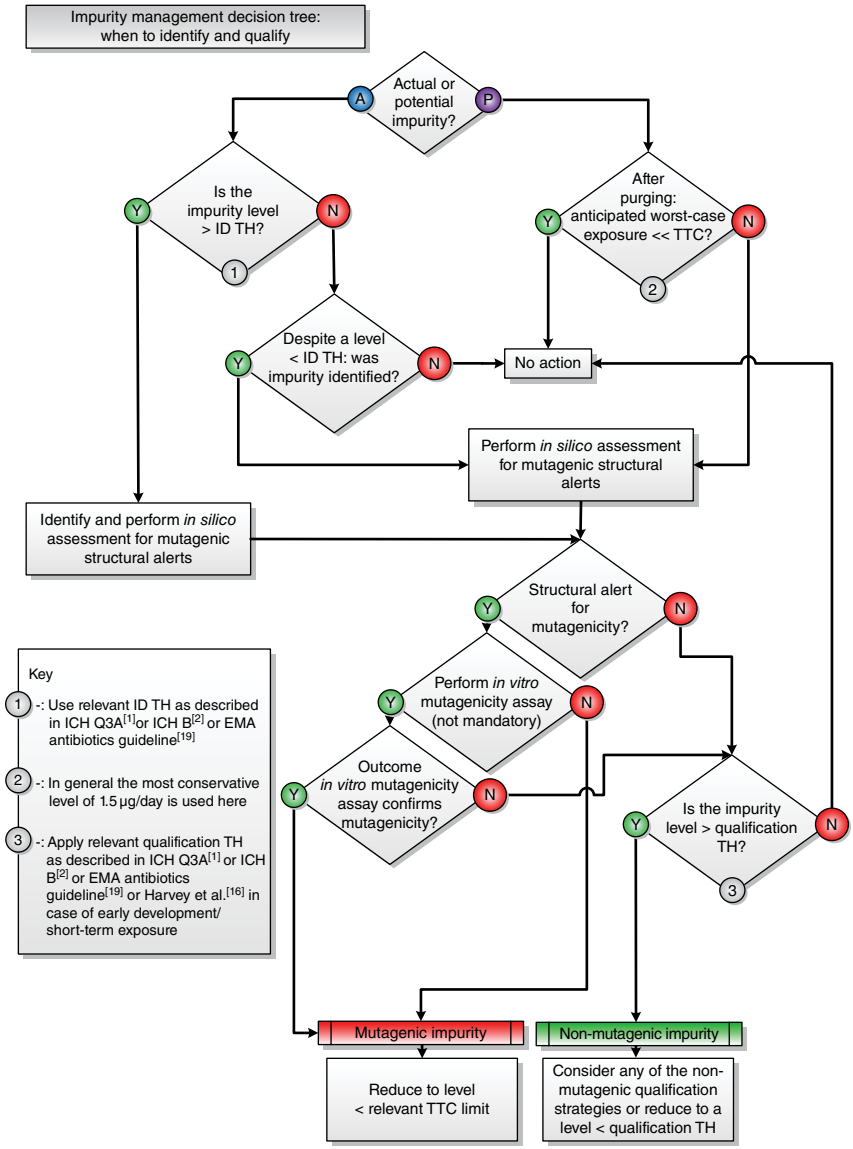

Impurity management decision tree: when to identify and qualify
Actual or potential impurity?
A
P
Is the impurity level > ID TH?
Y
N
1
After purging: anticipated worst-case exposure << TTC?
Y
N
2
Despite a level < ID TH: was impurity identified?
Y
N
No action
Perform in silico assessment for mutagenic structural alerts
Identify and perform in silico assessment for mutagenic structural alerts
Structural alert for mutagenicity?
Y
N
Perform in vitro mutagenicity assay (not mandatory)
Y
N
Outcome in vitro mutagenicity assay confirms mutagenicity?
Y
N
Is the impurity level > qualification TH?
Y
N
3
Key
1 -: Use relevant ID TH as described in ICH Q3A[1] or ICH B[2] or EMA antibiotics guideline[19]
2 -: In general the most conservative level of 1.5 µg/day is used here
3 -: Apply relevant qualification TH as described in ICH Q3A[1] or ICH B[2] or EMA antibiotics guideline[19] or Harvey et al.[16] in case of early development/ short-term exposure
Mutagenic impurity
Non-mutagenic impurity
Reduce to level < relevant TTC limit
Consider any of the non-mutagenic qualification strategies or reduce to a level < qualification TH

Figure 6.3 Adapted qualification process scheme incorporating details of ICH M7 and of other sections that may not be based on guidelines of the existing ICH Q3A and Q3B schemes.

Qualification should not be a process that hinders development of an appropriate and safe manufacturing process. In reality during drug development, there are distinct advantages to having higher levels of impurities in a DS in early development batches while still ensuring that purity is sufficient to

conclude that any efficacy/toxicity seen relates to the DS rather than impurities. The main reason for doing this is that it helps to qualify impurities early on at reasonable levels so that as process understanding and purity improves in later stages of development, the need for stand-alone qualification studies is minimized. Indeed it is somewhat of a fallacy to believe that reducing the levels of impurities to extremely low levels during early development will actually reduce testing. Regulatory guidelines dictate that preclinical toxicology studies are conducted prior to and in support of clinical use of a DS as well as ultimately registration of a new product. The assessment of impurity qualification is essentially a parallel activity, utilizing the data derived from these studies with the active. One significant risk to having very pure DS in early development is that any small changes in the impurity profile may result in a batch not being qualified for clinical use, leading to delays in time and cost, either for reprocessing of material or conduct of additional bridging toxicology studies.

Use of DS containing relatively high levels (but still safe) of impurities in early development helps to provide effective and comprehensive preclinical cover ensuring that clinical studies can proceed according to project plans and avoids the need for additional processing and/or toxicology studies.

6.6.2 Factors for Consideration in DS Purification

Purification is predominately achieved through recrystallization of the DS. It is important however that introduction of further purification steps is holistically assessed taking into consideration other key aspects. These include:

- Environmental impact
- Processing capability and manufacturing cost (ultimately impacting on the viability of the associated medicine)
- Impact on other quality attributes, for example, morphic form and hence the performance of the DP
- Other impurities including residual solvents (ICH Q3C(R5)) [3], elemental impurities (see ICH Q3D) and mutagenic impurities (see ICH M7) [5])

6.6.2.1 Environmental Impact

Introducing even an apparently simple recrystallization to reduce the level of an impurity has a significant environmental impact. The main issue is waste. Waste includes the loss of active; a typical recrystallization will produce 85–90% yield, and hence around 10–15% of the active becomes waste. Furthermore introduction of a final recrystallization with a yield of 85% increases the overall waste generated from the entire manufacture by approximately 20%.

A typical modern pharmaceutical manufacturing route might consume 200 metric tons of input materials for each ton of active produced following an

optimization exercise. This is a function of the complexity of the product and associated quality targets and will include contributory raw materials as well as water, solvents, precious metal catalysts, etc. For an active with an annual volume of 20 tons per annum (tpa), introducing an additional recrystallization step might equate to production of an additional 800 tpa of waste. However this could easily increase by an order of magnitude or more for higher volume products (e.g., cardiovascular) or for older, less efficient manufacturing routes. There is also the significant issue of the safety and effective disposal of the waste generated. All forms of disposal, for example, incineration/landfill, do themselves have an associated environmental "cost" and social challenges.

Energy costs are also associated with the material and waste costs. There is the increased cost of the energy directly consumed with the recrystallization. This may be particularly significant if the process involves heating a solvent to reflux. Secondly there is the overall increase in energy cost throughout the manufacture simply to address the loss of product arising from the additional purification stage.

6.6.2.2 Processing Capability

Recrystallization is often an efficient and effective process for the removal of impurities. There are however instances where this is not applicable. Clearly such a process relies on the isolation of a crystalline product and hence is not applicable to the purification of an amorphous solid or oil. Even for crystalline products, closely related impurities that differ from the desired product in only very minor ways, such as simple isomers, are often difficult to effectively remove as they exhibit very similar physicochemical properties. Multiple recrystallizations required under such circumstances are not viable due to amplification of the cost and environmental impacts, as described previously.

Other options such as chromatography are similarly impractical especially at a typical manufacturing scale. Chromatography uses large volumes of solvent(s), and while this can often be recovered (via energy-intensive distillation), the whole process is very time consuming. There is also insufficient global capacity for each active to be purified via this route.

6.6.2.3 Manufacturing Cost

Manufacturing cost is an important cost in the development of any new medicines; Figure 6.4 illustrates factors that affect this.

Within these, the chemical development of the DS can have a critical impact. Some areas include:

1) Route design. The design of the routes makes a positive contribution to reducing development costs. The more complex the DS, the greater the potential contribution to cost of goods (CoGs). Establishing a short route

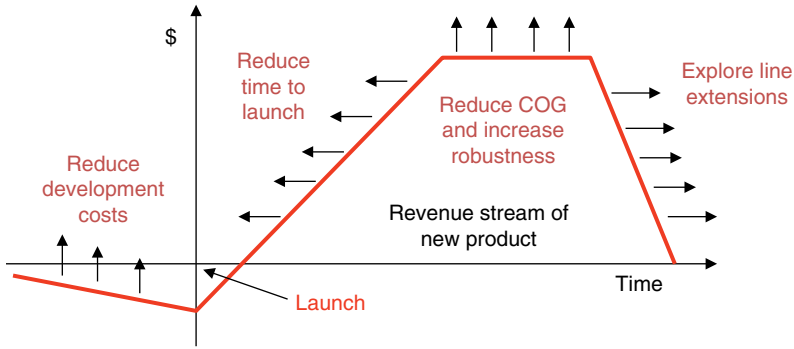


Figure 6.4 Illustration of factors that affect the revenue of a product.

impacts all aspects of the curve: it reduces cost during development, resulting in reduced CoGs and shorter manufacturing times, allowing us to make use of attrition; and fewer steps also increases productivity.

2) Process design (process development) also makes a positive contribution to development cost reduction; again the size varies depending on the complexity of the DS (complexity relating to the molecular weight of the molecule, the number of specific structural features, and the chemical process required to introduce the molecular framework and functionality). The delivery of robust and flexible processes is also critical to the long-term manufacture of the product.

Over the lifetime of the product, the contribution from optimization of route and process can be as high as a $1 billion.

However the additional financial impact associated with the introduction of an additional recrystallization may well threaten the economic viability of the medicine concerned. Introduction of a more economic manufacturing route generally allows multiple benefits—reduction in material costs through reduction of the levels of input materials, shorter manufacturing campaigns, and less waste for disposal—each of which contributes to a cheaper DS cost. Addition of wasteful steps such as a potential recrystallization of the active pharmaceutical ingredient (API) automatically increases the contributions from each of these costs since there is a pro rata need to process more of the "crude" material of current quality to deliver a slightly more purified active.

Another often discussed related topic is, "why do you change the process during development?" The initial routes and processes that are used to deliver DS are typically focused on the delivery of small samples of large numbers of compounds for initial evaluation in preclinical efficacy and safety studies. By definition, such processes are as generic as possible since speed is of the essence and high-throughput chromatography can be applied to improve quality.

While these approaches can often be scaled up with improvements, the routes are generally inefficient and not amenable to long-term operation. Furthermore, many of the reagents used at a gram scale are highly hazardous or largely unavailable at multi-kilogram scale. Identification and development of the best process route is a key part of chemical development. Key features of a scalable long-term route will be improved environmental performance, shorter manufacturing cycles, lower CoGs, better safety performance, and greater ability to accommodate in a manufacturing plant in a reliable and reproducible manner. Even during development such changes might remove 1000 tpa waste with annual savings >$1 million for a single project. Through the life of a DS until withdrawal from the market, this might equate to >105 tons waste and >$100 million savings as a consequence of introduction of a new route.

Changes in the route/process may also result in a change in the impurity profile that may drive the need for further evaluation, including additional preclinical toxicological qualification; however substantive qualification at an early stage helps to minimize this.

6.6.2.4 Impact of Recrystallization upon Physical Form

Changes in impurity profile can, in some instances, have a significant impact on the morphic form of the DS. Even parts per million levels of an impurity may promote/inhibit growth at a crystal face, resulting in isolation of an unwanted polymorphic form Ref. [22]. This again is a factor for consideration.

6.6.3 DS Control Strategy: Conclusions

The question as to "how pure should a DS be?" is a complex question requiring the consideration and balancing of a number of factors; these include quality, safety, economic, and environmental factors. The requirements for one factor may not align with that of another. A simplistic perspective based only on quality and "as low as reasonably practicable" (ALARP) principle is impractical as in many cases it would adversely impact the viability of the process and render the drug in question untenable.

Furthermore the introduction of excessive purification steps during early clinical development increases the risk that the impurity profile of subsequent DS batches is unrepresentative and is not qualified, leading to a potential increase in the need for additional preclinical testing and use of animals in contrast to an intended reduction.

Ultimately what is required is the development of a manufacturing process that delivers DS safely and consistently at scale to a quality proven safe through preclinical qualification. This must be matched with a process that is both economically and environmentally viable and that delivers DS of the required quality.

6.7 DP Purity: Key Points for Consideration

6.7.1 DP Degradation Pathways

DP degradation can in extremis result in inadequate shelf life or product recall. The mechanisms for API and DP degradation are fairly well understood, although it is by no means a fully "developed" science [23]. Comprehensive reviews of the two most commonly encountered degradation pathways in both API and DPs, that is, hydrolysis [24] and oxidation [25], are available in the literature. In silico strategies are also available to predict likely degradation pathways [22] combined with well-designed degradation studies, which allow the development of the optimal formulation, process, and pack configurations. In addition, modeling the role of water and temperature can be performed using Accelerated Stability Assessment Program (ASAP), and this again can be useful in defining the optimal formulation, process, and pack configuration [26, 27].

Of these two degradation mechanisms, oxidation is the most difficult to predict and hence the most difficult to control. This is because oxidation occurs via free radical-mediated mechanisms and the role of excipients [28] can often be key in destabilizing the API. For example, some excipients are auto-oxidizable in nature, that is, those containing the poly(oxy)ethylene structural motif, for example, polyethylene glycol (PEG), polysorbates, etc., and reactive impurities in these excipients can facilitate oxidative degradation. Control strategies are achievable but depend on a thorough understanding of the critical quality attributes (CQAs) and critical process parameters (CPPs). The control strategy can be subdivided into (i) control of excipients, (ii) control of formulation, (iii) control of process, and (iv) control of packaging.

6.7.2 Control of Excipients

Due to the intrinsic insolubility of most APIs, solubilizing excipients are required to facilitate solubilization, particularly for injectable DPs. However, many of the best solubilizing excipients, particularly surfactants and cosolvents, contain the poly(oxyethylene) structural motif that is susceptible to auto-oxidation. This oxidative cascade yields hydroperoxidyl and peroxidyl radicals, as well as short-chain aldehydes (e.g., formaldehyde and acetaldehyde) and their corresponding carboxylic acids (e.g., formic and acetic acid). These reactive impurities (particularly the radical species) can in turn cause oxidative instabilities in susceptible APIs [29] and can cause cross-linking of gelatin capsule products, particularly softgels [30]. In addition, these reactive impurities will vary according to supplier, batch, the storage conditions (open vs. closed containers), and the molecular weight—this is particularly true for PEGs [25]. The levels of these reactive impurities can often be appreciable. "Typical" levels of

volatile aldehydes and hydroperoxides have been reported for several common excipients [31, 32].

Kaisraian et al. [33] showed that residual peroxide levels in polyvinylpyrrolidone (PVP) could be directly linked with oxidatively mediated color changes in an injectable formulation. When levels of peroxide were controlled to <400 ppm, there was no discoloration seen. However, the authors did not explain how residual levels of peroxide were controlled to this limit (<400 ppm), and this can often be difficult. It is also unrealistic to expect that existing compendia limits for these reactive impurities provide adequate control [30]. In addition, setting specifications over and above compendial requirements is often challenging. The application of an in-use specification for every batch of an incoming excipient for a given DP is often difficult to manage. In addition, a tighter specification may only be specific for a particular product and will not necessarily represent the applicable limits for other products [30]. To address this need, several companies have introduced low aldehyde/low epoxide grades of common poly(oxyethylene) excipients. In parallel, some companies have elected to provide poly(oxyethylene) excipients with very small levels of an antioxidant, for example, butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT). Oxidation can be greatly influenced by the presence of catalytic levels of transition metals, but these can be difficult to control. It may be possible as part of ICH Q3D [4] screening processes to get more insight into residual metal levels in key excipients and assess whether alternative suppliers offer improved metal impurity profiles that could enhance oxidative stability [25]. Kaisraian et al. [33] showed that the levels of residual iron in a solubilizing agent varied significantly between vendors (A, 140 ppm; B, 13 ppm; C, 0 ppm), and this was directly correlated with an oxidatively induced color change in the formulation (A, significant color change; B and C, no color change). In this example, the authors indicated that the addition of a chelating agent was precluded by other formulation considerations. In this case a supplier-based control strategy was required.

6.7.3 Control of Formulation

As with all major degradation mechanisms, oxidation is greatly influenced by drug concentration. Therefore, the formulation should use the highest API drug loading [25] that is commensurate with flow, compression, and other processing parameters. Excipient compatibility will identify those excipients that are likely to adversely influence oxidative stability; however, there is often "play-off" between optimal stability, optimal processing, and optimal biopharmaceutical properties. Therefore, avoidance of excipients that promote oxidative instability may not be tenable. The formulation strategy therefore needs to address all of these competing criteria and arrive at a pragmatic outcome. This can include the following approaches.

6.7.3.1 Cosolvents/Surfactants

Poor solubility APIs often require the addition of poly(oxyethylene) excipients, for example, PEGs (cosolvents) or polysorbate 80 (wetting agents), to enhance solubility. This is critically important for sterile injectable formulations [34].

6.7.3.2 Chelating Agents

As oxidation is influenced by the presence of transition metals, the most favored formulation strategy is to add chelators to the formulation. These include polyfunctional carboxylic acids, for example, citric acid, malic acid, or ethylenediaminetetraacetic acid (EDTA). Unfortunately, it is not easy to predict which chelator will be most effective [22, 34].

6.7.3.3 Buffers

pH can often play a pivotal role in the stabilization of oxidatively labile drugs, and stability is often enhanced under acidic pH [30]. In oral liquid formulations, buffering to pH 4–5 is often preferable. Ephedrine injectable products formulated at pH 7.4 require the use of two antioxidants (acetylcysteine and ascorbic acid), whereas when formulated at pH 3.5, the product has no requirement for antioxidant stabilization [35]. In addition, pH can directly influence the performance of the antioxidant(s). In solid oral dosage forms, a similar rationale applies, but unlike solution formulations, the buffer must be nonvolatile, and this restricts the choice to carboxylic acids with pK_a in the region of 3–5.

6.7.3.4 Antioxidants

Selection of the most appropriate antioxidant(s) can be challenging and is often empirical in nature [29]. The most widely used antioxidants are the hindered phenols (BHA or BHT), which form stable radicals, leading to chain terminators or reducing agents (ascorbic acid, thiols (i.e., thioglycerol or thioglycolic acid), or polyphenols (i.e., propyl gallate)). They often work synergistically at very low concentrations, especially in liquids [35]; but higher concentrations may be required in solid oral dosage forms due to the lower molecular mobility in these systems [25]. Won et al. [34] reported that propyl gallate effectively stabilized one oxidative pathway of a candidate drug but not both. They also found that the antioxidant worked best with a chelator, that is, EDTA as the degradation pathway was metal ion catalyzed (Cu^{3+}). The most popular choice of antioxidants for sterile formulations is ascorbic acid, thiols (thioglycerol or thioglycolic acid), and sodium metabisulfite/sodium bisulfite. The hindered phenols (BHA, BHT) tend to be too insoluble to be used routinely in sterile solutions [35].

6.7.3.5 Cyclodextrins

Cyclodextrins are known to stabilize oxidatively labile drugs by protecting the structurally labile motif within the cyclodextrin torus [36]. However, CoGs or formulation constraints can be limiting factors [25] for such materials to be used.

6.7.4 Control of Process

It is good practice (particularly for liquid formulations) to either reduce or eliminate metal contact by using glass mixing vessels. This is particularly true for non-passivated stainless steel [22]. Oxygen-sensitive APIs in liquid formulations can be effectively protected from unwanted oxidation by introducing a nitrogen headspace during the manufacturing process. Several standard solid oral dose form manufacturing approaches can expose the formulation to elevated temperatures (hot melt granulation, fluid bed drying), high moisture (wet granulation, spray drying), or high pressure (roller compaction, tablet compression), which can destabilize certain APIs, leading to increased degradation. High shear mixing can destabilize oxidatively labile drugs. Similarly, an oxidatively labile drug showed increased destabilization during fluid bed granulation that was related to increases in hydroperoxide formation during processing, rather than input levels of peroxides [37]. The authors addressed these issues by introducing a control strategy that minimized hydroperoxide formation by controlling manufacturing parameters.

6.7.5 Control of Packaging

Oxidative degradation is thought to occur via auto-oxidative mechanisms involving both oxygen migration through the primary pack and headspace-derived molecular oxidation [38]. Therefore, the optimal selection of the container/closure with respect to minimizing oxygen ingress is appropriate. However, as with moisture vapor transmission rates (MVTR), there is a balance between cost and protection. Typically, the better the barrier protection afforded by the pack, the greater the cost.

However, the key vulnerability of any bottle presentation is the closure, and if screw-topped closures are used, then leakage can be significant. Therefore, when packaging oxidatively labile products, an oxygen-impermeable seal is typically used and induction heat-sealed closures can be very effective. Additionally, oxygen scavenger packaging inserts have been used to improve oxidative stability [39, 40]. Packaging with an inert gas blanket is logical and pragmatic option for minimizing oxidative degradation derived from headspace oxygen. Most sterile injectable products are packaged in glass ampoules or syringes or glass vials, with rubber plungers or closures. The plungers and closures tend to be the "weak point" of the container/closure system, and the best materials from an oxygen transmission perspective are halobutyl and butyl closures. Although glass containers have good permeability properties, they can still be a source of metal ion extractables/leachables [41]. There is <0.05% iron oxide in type I clear glass vials and between 1.0 and 1.2% of iron oxide in type I amber glass vials. Levels of manganese oxide in amber glass vials can be much higher, that is, 5–7%. Zinc and barium oxide can also be present. These metal ion leachables can often catalyze oxidation reactions [22]. In addition, alkaline

leachates from glass can enhance oxidation by increasing the pH; the latter can be addressed by ammonium sulfate treatment prior to use [42]. In addition, Kasraian et al. [33] indicated that light can also catalyze auto-oxidation reactions, which is why amber glass is often utilized for parenteral products. These authors also commented on the vial size, noting that generally larger vials provide a lower headspace-to-volume ratio than seen in smaller vials.

6.8 Conclusions

ICH guidelines ICH Q3A [1] and ICH Q3B [2] have been in place close to 20 years. Although there have been revisions, the central tenants of both guidelines, which are focused around a series of thresholds defining reporting/identification and qualification, remain essentially unaltered. Are they still fit for purpose?

Certainly there are flaws, considering that a more recent guideline (ICH M7) covers both developmental and marketing phases of products, while ICH Q3A and Q3B leave a gap regarding early development. Moreover, ICH M7 provides guidance on how to adjust limits in view of limited exposure periods, while the ICH Q3A and Q3B guidances do not. Some of these issues have been addressed in a recent publication of Harvey et al., which provides a scientific rationale for the initial lifelong safe limit of 1 mg/day for non-mutagenic impurities as well as an approach to derive shorter-term exposure limits and a proposal for early development limits [16].

Crucial is the key principle of the ICH Q3A guideline: the lifelong safe limit of 1 mg/day of an unqualified impurity is scientifically defendable based on the substantive safety data available. And that in the more recent ICH M7 guideline [5], the ICH Q3A and Q3B reporting and identification thresholds in principle have been reconfirmed. Therefore, especially applying modifications as proposed by Harvey et al. [16], generally the requirements defined by the ICH Q3A and Q3B guidelines, although dated, are still manageable and, with acceptance of proposed modifications, may actually lead to a lesser requirement for *in vivo* impurity qualification studies. It therefore seems reasonable to suppose that ICH Q3A [1] and ICH Q3B [2] will continue to play a seminal role in the overall management of impurities for the foreseeable future.

References

1 ICH Q3A(R2). 2006. Impurities in new drug substances. Current step 4 version, October 25, 2006.
2 ICH Q3B(R2). 2006. Impurities in new drug products. Current step 4 version, June 2, 2006.

3 ICH Q3C(5). 2011. Impurities: Guidelines for residual solvents. Current step 4 version, February 4, 2011.
4 ICH Q3D. 2014. Guideline for elemental impurities. Current step 4 version, December 16, 2014.
5 ICH M7. 2014. Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. Current step 4 version, June 23, 2014.
6 Teasdale A, Elder D, Chang S-J, et al. 2013. Risk assessment of genotoxic impurities in new chemical entities: Strategies to demonstrate control. *Organic Process Research and Development*, 17, 221–230.
7 ICH Q9. 2005. Quality Risk Management. Current step 4 version, November 9, 2005.
8 ICH Q2(R1). 2005. Validation of analytical procedures: Text and methodology. Current step 4 version, November 2005.
9 Diana F, Hofer J, Chatfield M, et al. 2014. Analytical method comparability in registration and post-approval stages: A risk-based approach. *Pharmaceutical Technology*, 38(10), 60.
10 Pohl M, Schweitzer M, Hansen G, et al. 2010. Implications and opportunities of applying the principles of QbD to analytical measurements. *Pharmaceutical Technology Europe*, 22(2), 29–36.
11 Dejaegher B, Jimidar M, De Smet M, et al. 2006. Improving method capability of a DSHPLC assay. *Journal of Pharmaceutical and Biomedical Analysis*, 42, 155–170.
12 ICH Q6A. 1999. Specifications: Test procedures and acceptance criteria for new drug substances and new DPs: Chemical substances. Current step 4 version, October 6, 1999.
13 Munro IC, Ford RA, Kennepohl E, et al. 1996. Correlation of structural class with no-observed-effect levels: A proposal for establishing a threshold of concern. *Food and Chemical Toxicology*, 34(9), 829–867.
14 Munro IC, Renwick AG, Danielewska-Nikiel B. 2008. The threshold of toxicological concern (TTC) in risk assessment. *Toxicology Letters*, 180(2), 151–156.
15 Tluczkiewicz I, Buist HE, Martin MT, et al. 2011. Improvement of the Cramer classification for oral exposure using the database TTC RepDose: A strategy description. *Regulatory Toxicology and Pharmacology*, 61, 340–350.
16 Harvey J, Fleetwood A, Ogilvie R, et al. 2017. Management of organic impurities in small molecule medicinal products: Deriving safe limits for use in early development. *Regulatory Toxicology and Pharmacology*, 84, 116–123.
17 Cramer GM, Ford RA, Hall RL. 1978. Estimation of toxic hazard: A decision tree approach. *Food and Cosmetics Toxicology*, 16, 255–276.
18 Gaylor DW. 2000. The use of Haber's law in standard setting and risk assessment. *Toxicology*, 149, 17–19.

19 EMA/CHMP/CVMP/199250/2009corr. 2012. Guideline on setting specifications for related impurities in antibiotics. Published June 30, 2012.
20 EDQM European Pharmacopoeia. 2010. Guide for the elaboration of monographs on synthetic peptides and recombinant DNA proteins. https://www.edqm.eu/medias/fichiers/NEW_Guide_for_the_elaboration_of_monographs_on_syn.pdf (accessed on April 7, 2017).
21 Norwood DL, Paskiet D, Ruberto M, et al. 2008. Best practices for extractables and leachables in orally inhaled and nasal drug products: An overview of the PQRI recommendations. *Pharmaceutical Research*, 25(4), 727–739.
22 Reid DL, Calvitt CJ, Zell MT, et al. 2004. Early prediction of pharmaceutical oxidation pathways by computational chemistry and forced degradation. *Pharmaceutical Research*, 21, 1708–1717.
23 Baertschi SW, Alsante KM, Reed RA 2011. *Pharmaceutical stress testing: Predicting drug degradation*, Informa Healthcare, London.
24 Waterman KC, Adami RC, Alsante KM, et al. 2002. Hydrolysis in pharmaceutical formulations. *Pharmaceutical Development and Technology*, 7, 113–146.
25 Waterman KC, Adami RC, Alsante KM, et al. 2002. Stabilization of pharmaceuticals to oxidative degradation. *Pharmaceutical Development and Technology*, 7, 1–32.
26 Waterman KC, Carella AJ, Gumkowski MJ, et al. 2007. Improved protocol and data analysis for accelerated shelf-life estimation of solid dosage forms. *Pharmaceutical Research*, 24, 780–790.
27 Waterman KC. 2011. The application of accelerated stability assessment program (ASAP) to quality by design (QbD) for DP stability. *AAPS PharmSciTech*, 12, 932–937.
28 Hovarka S, Schöneich C. 2001. Oxidative degradation of pharmaceuticals: Theory, mechanism and inhibition. *Journal of Pharmaceutical Sciences*, 90, 253–269.
29 Hartauer KJ, Arbuthnot GN, Baertschi SW, et al. 2000. Influence of peroxide impurities in povidone and crospovidone on the stability of raloxifene hydrochloride in tablets: Identification and control of an oxidative degradation product. *Pharmaceutical Development and Technology*, 5, 303–310.
30 Wu Y, Levons J, Narang AS, et al. 2011. Reactive impurities in excipients: Profiling, identification and mitigation of drug-excipient incompatibility. *AAPS PharmSciTech*, 12, 1248–1263.
31 Li Z, Kozlowski BM, Chang EP. 2007. Analysis of aldehydes in excipients used in liquid/semi-solid formulations by gas chromatography-negative chemical ionization mass spectrometry. *Journal of Chromatography A*, 1160, 299–305.
32 Wayslaschuck WR, Harmon PA, Wagner G, et al. 2007. Evaluation of hydroperoxides in common pharmaceutical excipients. *Journal of Pharmaceutical Sciences*, 96, 106–116.

33 Kasraian K, Kuzniar AA, Wilson GG, et al. 1999. Developing an injectable formula containing an oxygen sensitive drug: A case study of Danofloxacin injection. *Pharmaceutical Development and Technology*, 4, 475–480.

34 Won CM, Tang S-Y, Strohbeck CL. 1995. Photolytic and oxidative degradation of an antiemetic agent, RG 12915. *International Journal of Pharmaceutics*, 121(1), 95–105.

35 Ackers MJ. 2010. Chapter 6: Formulation components (solvents and solutes). Chapter 8: Formulation and stability of solutions. In: *Sterile drug products*, Informa Healthcare, New York, London, pp. 67, 101.

36 Duchene D, Debrueres B, Vaution C. 1985. Improvement of drug stability by cyclodextrin inclusion complexation. *Sciences Techniques et Pratiques Pharmaceutiques Sciences*, 1, 37–43.

37 Harman PA, Wuefling WP, Harman AB, et al. 2004. *Role of organic hydroperoxides on process robustness and finished product quality*. Presented at AAPS, November 7–11, 2004, Baltimore Convention Center, Baltimore.

38 Mahajan R, Templeton A, Harman A, et al. 2005. The effect of inert atmospheric packaging on oxidative degradation in formulated granules. *Pharmaceutical Research*, 22, 128–140.

39 Waterman KC, Roy MC. 2002. Use of oxygen scavengers to stabilize solid pharmaceutical dosage forms: A case study. *Pharmaceutical Development and Technology*, 7, 227–234.

40 Waterman KC, MacDonald BC. 2010. Package selection for moisture protection for solid, oral DPs. *Journal of Pharmaceutical Sciences*, 99, 4437–4452.

41 Pavanetto F, Gento I, Conti B, et al. 1989. Investigation on the contribution of raw materials and of the primary container on aluminum, cadmium and lead contamination of large-volume parenteral solutions. *International Journal of Pharmaceutics*, 54, 143–148.

42 Roseman TJ, Brown JA, Scothorn WW. 1976. Glass for parenteral products: A surface view using the scanning electron microscope. *Journal of Pharmaceutical Sciences*, 65, 22–29.

7

ICH Q3C Impurities

Guideline for Residual Solvents

John Connelly

ApoPharma Incorporated, Toronto, Ontario, Canada

7.1 Introduction

Until the advent of the ICH Q3D document *Guideline for Elemental Impurities* [1], the ICH Q3C guideline [2] was unique among ICH's output in establishing individual limits for a series of named impurities, that is, residual solvents, or "organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products." This approach has both advantages and drawbacks. Beneficially, it provides clear and unequivocal guidance on the amounts of listed solvents that a patient can be exposed to as residues in a pharmaceutical product, unless the manufacturer can fulfill the responsibility of justifying higher intakes. A limitation is that it relies heavily on the availability of very specific data sets. There is no process allowing use of analogy and extrapolation of comprehensive and reliable information on one solvent to facilitate derivation of a limit for "related" ones. If a solvent is used in pharmaceutical manufacturing, warranting incorporation of a limit in ICH Q3C, sufficient relevant toxicological data on that molecule to support the limit must be identified or generated. In this respect, it was also recognized early in the development of the guideline that more information on solvents already in the lists would be forthcoming, or that new solvents would need to be added. Accordingly, a maintenance procedure was put in place (see Section 7.7), but scant awareness and understanding of it, and the resource required to obtain the required data, evidently contribute to its underutilization.

The ICH Q3C guideline assigns residual solvents in pharmaceuticals to classes based on measures of their toxicity but has resisted gross and inconsistent "rounding" of the daily limits ("permitted daily exposures" or PDEs) to tidy

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up the numbers. The resulting lack of artificially convenient groups of materials with numerically the same limits has inherent virtues and faults. On the one hand, the discrete properties of the compounds, and of the data set underlying calculation of the PDE, remain emphasized. On the other hand, there is a risk of misleading users into taking for granted a degree of precision of the values that is not justified by the actual data set. One cannot reasonably use biological data that may be old, incomplete, and less than perfectly collected or collated, or in a significant number of cases all of these, to conclude that the boundary of acceptance and rejection for example, *N*,*N*-dimethylacetamide (*N*,*N*-DMA) is exactly 10.9 mg/day or of N-methylpyrrolidone is 5.3 mg/day. Nonetheless, there is no particular gain in making the PDEs 10.0 and 5.0 mg/day except for the human tendency to be reassured by "round numbers."

Any guideline that is the product of multiple parties with a common aim but differing remits is a compromise. Self-evidently, ICH Q3C is a quality guideline, but it is based on solvent safety, and it required toxicologists, chemists, analysts, and QA experts to learn each other's languages and to split the awkward difference of actual "dose" (toxicologists) and concentration (everyone else). As many solvents as possible that were important to the industry had to be included, in the face of publications of varying completeness, quality, and idiosyncrasy, but without compromising safety or the fundamental principles of the guideline. While complete objectivity might have been desirable, ultimately elements of safety experts' judgment had to be applied to determine, for example, whether solvents with borderline data sets should or should not be listed. Missing study types, incomplete details of design, seemingly eccentric dose level selection, and failure to identify clear no-observed-effect levels (NOELs), all added their quota to the practical decision-making needed to deliver the ICH Q3C guideline. In many respects ICH Q3C is an enduring tribute to pragmatism; it is imperfect, but it has worked sufficiently well to serve both industry and regulators for 17 years since step 4.

7.2 Objective and Scope of the ICH Q3C Guideline

The aim of the ICH Q3C guideline is to define the levels of named solvents that patients can be exposed to with a reasonable certainty of not compromising their safety and to express that exposure in the context of residues in pharmaceutical products. Because safety is the guiding principle, the key expression is of the *amount* of a particular solvent to which a patient can be exposed daily; this amount is converted into a concentration in a fixed daily dose of product to provide consistency with analytical concepts.

Solvents are a necessary element of drug substance, drug product, and excipient manufacture, and their directed use helps to govern a variety of critical attributes of the final product, including yield, polymorphism, purity, impurity

profile, solubility, etc. At the same time, residual solvents are of no benefit to patients (and may, e.g., accelerate product degradation), but it is neither practical nor necessary from a safety perspective to target zero levels. Hence, intakes via drugs are controlled to those considered to be safe, unless quality-based limits intervene in the case of solvents of lower toxicity.

The solvents listed in ICH Q3C are examined first, individually, via a procedure aimed at determining maximum permissible daily exposures, using published animal toxicity studies as the primary data source. They are then classified according to a scheme that recognizes those that should be avoided other than under exceptional and justified circumstances, those with toxic properties that require specific limitation, and those for which quality considerations predominate. Limits can be revised if new data come to light (and potentially alter the originally assigned classification), and other solvents added to the guideline. Furthermore, levels of a solvent not included in the guideline may be supported on an individual case basis by applying ICH Q3C concepts in a marketing authorization submission, as well as by using other impurity guidance documents. This is examined in more detail in the following text.

The scope of the guideline encompasses residual solvents in drug substances, excipients, and drug products formulated from them. Solvates, and solvents used as components of products, are excluded. ICH Q3C gives a reminder that the solvent levels in such products should be evaluated and justified, but anomalies are inevitable. The ethanol content of many cough syrups provides a quantity to the patient markedly exceeding that nominally permitted by ICH Q3C of 50 mg/day (5000 ppm in 10 g drug product), the guideline limit being defined by quality concepts alone. Amounts normally taken therapeutically would be trivial compared with even modest social alcohol consumption. Arguments for the high ethanol content of relevant medicines can be made on a case-by-case basis, although one has to wonder whether ethanol might have been more reasonably excluded from the ICH Q3C list, or given special treatment.

As written, the guideline states that it is not applicable to investigative drug products or their components during the clinical research phases of development, or retrospectively to marketed products. Nonetheless, regulatory comments arise not uncommonly with respect to both of these situations. The author of this chapter has been asked more than once at IND and phase 2 clinical trial stages of development "If we don't apply ICH Q3C (or ICH Q3A, or ICH Q3B, as appropriate), what *should* we apply in the interests of patient safety?" The unspoken—or sometimes, spoken—question is "Are clinical trial patients less worthy of safe treatment than those exposed after marketing?" The answers demand some thought, which is better applied up front if one is vulnerable to such interrogation. Similar issues may occur in the case of approved products when, for example, unrelated amendments to specifications or routine updates provide a regulatory agency with the opportunity to probe residual solvent levels.

All dosage forms and routes of administration are covered by the limits set; this is considered further in Section 7.3. It is noted in ICH Q3C that higher residual solvent levels may be acceptable for short-term (30 days or less) or topical use, with justification, but this point reads as a passing comment and is not developed further. Caution is advised: the nature of the toxicity associated with the solvent of interest should be assessed, as should the comprehensiveness of the database. Not all adverse effects depend on long-term dosing, and solvents may pass readily through the skin.

7.3 Permitted Daily Exposure and Concentration Limits

The PDE[1] is the critical limit, as it defines the actual intake of a solvent permitted daily based on safety considerations, and therefore complies with the fundamental toxicological principle of "the dose makes the poison." However, in order to accommodate analytical perspectives, a second concentration-orientated approach is allowed for by the ICH Q3C guideline. The latter (denoted as option 1) is given by

$$\text{Concentration}\left(\text{ppm}\right) = \frac{1000 \times \text{PDE}\left(\text{mg/day}\right)}{10\left(\text{g/day}\right)}$$

The resulting limit applies to all drug substances, drug products, or excipients. If the solvent concentration measured in the product, or in each component individually, meets the limit, the need for further calculation is precluded provided that the maximum daily dose (MDD) of the product is 10 g or less. If the MDD exceeds 10 g, option 2 should be used.

Option 2 essentially considers the situation where not all components of a formulated product meet the concentration limit, but the MDD is less than 10 g/day. Entering the *actual* MDD and the PDE for a given solvent in the equation earlier provides the concentration of that solvent that can be allowed in the product, which can be demonstrated analytically. Alternatively, if the solvent concentrations in the drug substance and relevant excipients are known, the contribution of each to daily intake of the solvent can be calculated, summed, and shown to be less than the PDE. According to the ICH Q3C guideline, application of option 2 requires that limits meet manufacturing and

1 Various terms for safety-based limits of exposure to toxic industrial chemicals, food additives, environmental contaminants, and so on were already in use when the ICH Q3C guideline was composed. Although there was some reservation about coining another, the concept of the "permitted daily exposure" or PDE was adopted to prevent the same term taking different values (e.g., where a residual solvent also had an "acceptable daily intake" (ADI) assigned by WHO-JECFA) or to avoid inappropriate use where existing terms clearly or tacitly addressed specific routes of exposure or encompassed deliberately added materials, whereas residual solvents in drug products are regarded as of no benefit to the patient.

analytical standards and capability, that is, an MDD of less than 10 g does not grant freedom to exploit the PDE if this means that the resulting residual solvent concentrations do not comply with reasonable quality-based expectations.[2] These concepts are explored through a series of examples.

7.3.1 Examples

1) A drug product, MDD 4.0 g, contains the active substance and three excipients, wherein two have a residual acetonitrile (ACN) content while the third is free of ACN. The overall composition is listed:

Component	Amount in formulation (g)	Residual acetonitrile concentration (ppm)	Daily exposure (mg)
Drug substance	0.5	1000	0.50
Excipient 1	0.8	2500	2.00
Excipient 2	2.3	600	1.38
Excipient 3	0.4	0	0
Drug product	4.0	970	3.88

   The PDE for ACN is 4.1 mg/day, and the maximum concentration in a product of up to 10 g MDD is 410 ppm. Other than excipient 3, none of the components meet the option 1 limit, and the product contains more than 410 ppm ACN. Notwithstanding this, the daily amount of ACN to which the patient is exposed is less than the PDE and is acceptable, provided that quality standards are met.
2) Another drug product, MDD 4.0 g, also contains the active substance and three excipients, in this case all with ACN residues:

Component	Amount in formulation (g)	Residual acetonitrile concentration (ppm)	Daily exposure (mg)
Drug substance	0.5	1000	0.50
Excipient 1	0.8	2500	2.00
Excipient 2	2.3	800	1.84
Excipient 3	0.4	300	0.12
Drug product	4.0	1115	4.46

2 Note, however, that an EMA Q & A publication, Quality of medicines questions and answers: Part 1 http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/quality_qa_part1.jsp&mid=WC0b01ac05801bf0c2#section10 (accessed September 21, 2014) states that class 2 solvent residues can be present at up to ICH Q3C limits without additional tightening of the specification founded on batch data, namely: *For class-2 residual solvents in active substances or medicinal products, it is sufficient to restrict the specification to the concentration limit (parts per million) as defined in the notes for guidance on impurities: residual solvents (CPMP/ICH/283/95 & EMEA/CVMP/511/03) and related maintenance guidelines or to calculate it based on the respective permitted daily exposure (mg/day) outlined in the guidelines. It has been agreed by the QWP that there is no need for further tightening of the specification in line with batch results, as the limits in the guideline are based on safety assessment.*

In this instance, excipient 3 still meets the option 1 limit, but neither the other individual components of the formulation nor, critically, the final drug product does so. Although the MDD for the product is well under 10 g, the calculated ACN intake exceeds the PDE of 4.1 mg, and the product is not acceptable. Next steps open to the manufacturer would be to (i) analyze the product to establish whether solvent loss during formulation processes reduces the actual ACN level to less than 410 ppm; (ii) further purify the drug substance, change an excipient source, etc.; or (iii) justify the existing ACN concentration according to the principle that the level has been reduced to one that is as low as reasonably practicable based on cleanup and that the product has an adequate benefit: risk ratio.

Note that even the apparently simple rules applying to the two options do not prevent both real-life problems and theoretical questions from arising. It is natural to assume that a patient will take his or her prescribed dose of a medicinal product in the form of the highest available appropriate strengths, for example, that for Product X manufactured as tablets containing 20 or 100 mg of active substance, a 300 mg daily dose will consist of 3×100 mg tablets, not 15×20 mg tablets. Nonetheless, the latter scenario cannot be entirely excluded, and it is possible that a PDE may be met by the higher strength tablets but exceeded by the lower strength, because of the levels of a residual solvent in an excipient if the weights of the components of the formulations are not proportional. Another potential situation is the existence of a solvate, typically of the drug substance. Issues such as these might well prompt the question of why an alternative solvent is not selected, but that may not be feasible if, for example, the one used is critical to isolation of the drug substance in the desired physical form.

7.4 Solvent Classification

Assignment of solvents to classes based on their perceived level of risk is demanding, and subject to both real and apparent anomalies. Nonclinical data are critical to the process, as well as the source of most of the uncertainty. A study may be in good standing to all intents and purposes, but the PDE it yields may be substantially lower than those estimated from several other studies of the toxicity of the same solvent (see the example of toluene in the following text). The doses that happen to have been selected by the investigators may have a marked influence, particularly when their consequences are amplified by ICH Q3C safety factors. Subjective assessment of the importance of the toxicity noted at effect doses also plays its part, as does the presence or absence of an NOEL. Data quality invokes debate: papers describing the effects of solvents in animals may have been published many decades ago, with aims in mind that may or may not be apparent, and/or subject to bias. It may not be entirely clear whether the effects reported reflect a limited assessment, or an

analysis of a comprehensive range of end points. Highly detailed investigations present a challenge: findings at the cellular or molecular level, or subtle behavioral changes identified using special evaluation techniques, are often difficult to interpret and are effectively impossible to compare with the results of studies in which, for example, tissues are sectioned, stained routinely, and examined microscopically. Such specialized studies, if taken into account, may have a profound and perhaps unwarranted impact on a PDE, and hence possibly on classification. Should these studies be considered during derivation of a PDE? If so, how should safety factors be applied to the findings?

Notwithstanding these difficulties, the solvents included in the guideline were allocated to classes on the basis of available information.

7.4.1 Class 1 Solvents: Solvents to Be Avoided

Broadly, these include known and strongly suspected human carcinogens, and two compounds recognized as environmental hazards (Table 7.1). One of these solvents, 1,1,1-trichloroethane (1,1,1-TCE), appears at first glance to be misclassified, but the key message of class 1 is that use of its members should be avoided in the manufacture of drug substances, excipients, and drug products, and the rationale is applied to 1,1,1-TCE as it is an ozone-depleting agent. Rather ironically, it is the subject of an unusual wealth of toxicity studies, which are the source of the ICH Q3C limit of 1500 ppm. Note that only concentration limits are provided for class 1 solvents; again this reflects that their employment is strongly discouraged,[3] that is, "permitted daily exposure" would deliver an inappropriate message. However, the lack of PDEs for class 1 solvents occasionally causes logical inconsistencies. The guideline acknowledges that utilization of class 1 solvents in the manufacture of a particularly important drug

Table 7.1 Class 1 solvents included in Q3C(R5).

Solvent	Concentration limit (ppm)	Principal safety concern
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

3 EMA/CVMP/450/03—Rev. 1. Annexes to CPMP/ICH/283/95 Impurities: Guideline for residual solvents & CVMP/VICH/502/99 Guideline on impurities: residual solvents, February 20, 2013 states:
The use of a class 1 solvent in the manufacture of a finished product is not considered acceptable.

product may be required. If, in such a scenario, 1,1-dichloroethane (limit 8 ppm) was the solvent involved, it is at least arguable that 80 ppm in a 1 g/day product should be defensible on the basis that it would lead to exactly the same intake of 1,1-dichloroethane, 0.08 mg/day, as 8 ppm present in a product given at 10 g/day. Conversely, while allowing that pharmaceutical products given at more than 10 g/day are rare, in that instance the guideline would effectively permit exposure to the solvent to exceed the PDE from which the concentration limit was derived. A challenge to the acceptability of 8 ppm 1,1-dichloroethane in the product could then be foreseen, even if use of the solvent was otherwise justifiable.

7.4.2 Class 2 Solvents: Solvents to Be Limited

The list of class 2 solvents (Table 7.2) is pivotal to the guideline, as many are in common use in pharmaceutical manufacturing processes. Evaluation of the literature showed them to have toxicity profiles that mandate control of residual levels. This combination of circumstances suggests that it is with respect to class 2 solvents that it is particularly worth understanding something of the principles, gaps, conflicts, and elements of judgment that underlie PDE values, and the methods that would be employed in revising PDEs or in proposing additions to the lists.

The nature and severity of the adverse findings in animal studies vary substantially and involve a range of outcomes extending from evidently non-genotoxic carcinogenesis (a genotoxic carcinogen would be a candidate for class 1) through critical organ system damage (e.g., CNS, sensory, reproductive) that in some cases may be irreversible to renal, hepatic, hemopoietic, endocrine, and other target organ and system effects. Solvents associated with harm to the fetus are also in class 2. Studies designed to identify the functional reproductive toxicity of solvents, affecting fertility or postnatal development, have more rarely been reported.

Data packages available for class 2 solvents differ considerably in quantity and quality, to the extent that in certain cases decisions to retain solvents important to the industry in the ICH Q3C list have been the borderline ones. For example, neither adequate genotoxicity nor rodent carcinogenicity bioassay data could be located for several members of class 2, and judicious assessments of general toxicity study designs were made accordingly to determine whether a PDE could be assigned with sufficient confidence. In these cases a large aggregate safety factor may have been applied, resulting in a lowered limit. Typically, solvents are administered by inhalation in nonclinical studies, reflecting occupational exposure. Most pharmaceutical products are dosed orally, and the unavoidable extrapolation of findings across routes as well as species may distort conclusions. Clearly, absorption across the lung surface

Table 7.2 Class 2 solvents included in Q3C(R5).

Solvent	PDE (mg/day)	Concentration limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cumene	0.7	70
Cyclohexane	38.8	3880
1,2-Dichloroethene	18.7	1870
Dichloromethane	6.0	600
1,2-Dimethoxyethane	1.0	100
N,*N*-Dimethylacetamide	10.9	1090
N,*N*-Dimethylformamide	8.8	880
1,4-Dioxane	3.8	380
2-Ethoxyethanol	1.6	160
Ethylene glycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000
2-Methoxyethanol	0.5	50
Methylbutyl ketone	0.5	50
Methylcyclohexane	11.8	1180
N-Methylpyrrolidone	5.3	530
Nitromethane	0.5	50
Pyridine	2.0	200
Sulfolane	1.6	160
Tetrahydrofuran	7.2	720
Tetralin	1.0	100
Toluene	8.9	890
1,1,2-Trichloroethene	0.8	80
Xylene[a]	21.7	2170

[a] Usually 60% *m*-xylene, 14% *p*-xylene, 9% *o*-xylene with 17% ethylbenzene.

may produce systemic exposure and toxicity differing from that after oral or parenteral administration. These and other limitations explain, if not entirely validate, the relatively unsophisticated use of a safety (uncertainty) factor approach and the substantial denominators employed in setting PDEs. For

some class 2 solvents, such as dichloromethane, there is a wealth of information that would allow ADME, toxicological, and physiological elements to be blended into detailed models for cancer risk assessment, albeit much of that has emerged since step 4 of ICH Q3C's development in 1997. Some other solvents require much literature—and, sometimes, soul—searching in order to offer a PDE at all. Should the "best" (most sophisticated) procedure be adopted for each solvent, predicated on the data set available? The current approach has won out, for reasons of consistency and simplicity, and, not least, resource availability. However, that should not prevent parties proposing the addition of a new solvent to the guideline from applying both the established principles and alternative, appropriate methods to derivation of a PDE. If the latter results in a higher limit and other considerations are supportive, then arguing the case seems reasonable.

7.4.3 Class 3 Solvents: Solvents with Low Toxic Potential

Class 3 contains those solvents (Table 7.3) that show essentially no evidence of risk to human health at levels normally found acceptable in pharmaceutical products according to quality considerations. As such, intakes of 50 mg/day or less (5000 ppm or 0.5% in 10 g product under option 1) would be accepted without further justification. Higher amounts, if consistent with manufacturing capability and GMP, could be defended in a safety context by reference to the PDE. PDEs of class 3 solvents are derived in the same way as those of class 2 but exceed 50 mg/day.

Table 7.3 Class 3 solvents included in Q3C(R5).

Acetic acid	Heptane
Acetone	Isobutyl acetate
Anisole	Isopropyl acetate
1-Butanol	Methyl acetate
2-Butanol	3-Methyl-1-butanol
Butyl acetate	Methylethyl ketone
t-Butylmethyl ether	Methylisobutyl ketone
Dimethyl sulfoxide	2-Methyl-1-propanol
Ethanol	Pentane
Ethyl acetate	1-Pentanol
Ethyl ether	1-Propanol
Ethyl formate	2-Propanol
Formic acid	Propyl acetate

7.5 Estimating PDE Values: Safety Factors, Allometric Scaling, and Calculation Adjustments

7.5.1 General Principles

In pioneering a guideline attempting to set specific limits for named compounds used industry wide in pharmaceutical product manufacture, the ICH Q3C EWG had to consider the degree of granularity to be adopted in recognizing that medicines may be given by any of multiple therapeutic routes and for periods ranging from a day to a lifetime. The "ideal" document might address all of these discretely, but for many solvents important or critical to the industry, it was highly unlikely that the published literature would supply data supporting such an approach. The principle chosen was one of assuming lifetime exposure and combining a sufficient safety margin to allow all conventional therapeutic routes to be covered by a single PDE. This could be done by using long-term animal studies where they were available and by applying more substantial safety factors to shorter-term studies where they were not. This approach was overlaid on the assumptions that use of pharmaceuticals is necessary for a subset of the population, that solvents are employed in product manufacture and for practical purposes generally cannot be entirely removed, and that levels exceeding guideline limits must be strongly justified. Finally, a worthy but realistically impracticable target was that animal studies on which PDEs were based should always comply with authoritative protocols (OECD, EPA, FDA, etc.); much effort was made to evaluate the quality of published work, but information inevitably reflected the age, source, and original objectives of the studies, and therefore demanded considerable subjective judgment.

7.5.2 Class 1 Solvents

Solvents assigned to class 1 (Table 7.1) comprise those that are highly toxic, cause genetic damage in mutagenicity assays, are suspected human carcinogens, or have major environmental liabilities. For the carcinogenic compounds, either a risk assessment method such as the Gaylor–Kodell linear interpolation technique [3] or application of a very large safety factor to a study NOEL is recommended. PDEs of 0.04 and 0.08 mg/day for carbon tetrachloride and 1,1-dichloroethene, respectively, were derived by dividing a NOEL (CCl_4) and lowest observed effect level (LOEL) (1,1-DCE) from mouse carcinogenicity studies by a composite factor of 12,000. Evidence for the genotoxicity of carbon tetrachloride is less than overwhelming, and a chronic cycle of hepatocellular damage and proliferation may underlie the liver tumors reported in animals. However, CCl_4 is highly toxic in other respects and has marked ozone-depleting potential. Estimates of an excess cancer risk of 10^{-5} using multistage models yielded PDEs of 0.02 mg/day for benzene and 0.05 mg/day for

1,2-dichloroethane. 1,1,1-Trichloroethane, which has been tested in many genotoxicity assays with only limited evidence of effect, is atmospheric ozone depleting; a PDE of 15.0 mg/day is the result of a safety factor-dependent calculation. While a PDE process was followed for class 1 solvents, allowing concentration limits in a nominal 10 g/day dose of drug product to be calculated (Table 7.1), only the latter are listed in ICH Q3C. As indicated earlier, this is in accordance with the "message" that class 1 solvents are to be avoided, unless their use can be justified as the sole means of manufacturing a drug product that represents a significant therapeutic advance.

7.5.3 Class 2 Solvents

The "safety" or "uncertainty" factor approach that is the basis of the calculation of PDE values in the ICH Q3C guideline is of most applicability to class 2 solvents. The principle is widely used, most credibly when data on the characteristics of dose–response relationships, the biological phenomena determining those relationships (including threshold and saturation of metabolism) and interspecies differences, are limited. The factors used tend to be conservative and do little or nothing to take into account mechanistic and toxicokinetic information. Consideration was given to more sophisticated risk estimation methods early in the development of ICH Q3C, but the somewhat surprising lack of comprehensive studies of the safety, absorption, distribution, and metabolic fate of common solvents that have been in widespread use for many decades would make this impractical for all but a small number. It might also be expected that human data would be employed to a greater extent. However, most reports of toxicity in humans are little more than anecdotal and depend on frank signs of effect, or on the story told by a limited range of preselected markers. Tissue damage and subtle behavioral changes are readily missed. Quite properly, high doses, and toxicity that is evident only after long-term exposure, are rarely investigated prospectively. Therefore, derivation of PDEs relies largely on observations in animals, and key questions revolve around what type or duration of study constitutes an adequate package, the evaluation and acceptable standards of quality of data, the relative importance of different end points, and the most practical and appropriate means of extrapolating risk from multiple nonclinical species to humans.

The basic equation for calculation of the PDE for a solvent in ICH Q3C is

$$\mathrm{PDE}\left(\mathrm{mg/day}\right) = \frac{\mathrm{NOEL}\left(\mathrm{mg/kg}\right) \times \mathrm{weight\ adjustment}\left(\mathrm{human\ body\ wt\ in\ kg}\right)}{\mathrm{F1} \times \mathrm{F2} \times \mathrm{F3} \times \mathrm{F4} \times \mathrm{F5}}$$

A NOEL from an appropriate study (oral, inhalation, parenteral; preferably long-term general toxicity or carcinogenicity) in animals is the preferred foundation of a PDE; if more than one such study is available, generally the lowest NOEL is selected. In the absence of an NOEL, a LOEL may be used, although

subjective judgment will then be required to modify a safety factor (F5) according to the nature and severity of the finding determining the LOEL and the sum of the characteristics of the dose–response relationship.

No adjustment to the calculation is made for route of administration. Solvents commonly are readily absorbed into the systemic compartment of the body, including after inhalation and, often, dermal administration; essentially, 100% absorption is assumed.

7.5.3.1 Safety Factors

F1 is the modifying factor that addresses extrapolation from animal species to humans. Originally it was taken to be 10 in all cases but was modified subsequently to incorporate an element of interspecies scaling. This uses the approximate dependency of body surface area on body weight to the 2/3 power, that is:

$$\text{Surface area}\left(S\right) = \text{kM}^{0.67}$$

If body mass (M) is expressed in kg and $k = 10$, as in ICH Q3C, the value derived for S will be in dm^2. Dividing by 100 gives surface area in terms of m^2. Strictly speaking, the constant, k, will differ with species, although a common value of 10 provides sufficient precision for the guideline's purposes. The factors themselves are multiples of the human surface area to body weight ratio for a person weighing 50 kg. For example, the S : M ratios calculated for a person of this weight and a mouse of 28 g are 0.0275 and 0.1326, respectively; 0.1326/0.0275 = 11.8, which is adjusted to 12. The equation yields the following values of F1 for common laboratory species:

Species	Mouse	Rat	Rabbit	Dog	Monkey
Value of F1	12	5	2.5	2	3

F1 = 3 is assumed for all species of monkeys; this is reasonable for cynomolgus monkeys, which are commonly chosen for toxicity studies, but less suitable for the much smaller marmoset or larger rhesus macaque. An arbitrary value of 10 is assumed for species not listed. Naturally, the exact value of the factor will vary with the "representative" body weight chosen, but the close similarities to the divisors converting animal dose (mg/kg) to the human equivalent dose, as given in FDA guidance to first-in-human clinical trials [4], are clear.

F2 is included to allow for the variability in response among individuals and is given the value 10. There has been considerable debate about the range of sensitivity to biological effects across an entire population and it probably exceeds one order of magnitude, but experience suggests that a divisor of 10 will cover the great majority of subjects.

F3 is assigned a value according to the duration of the study on which the calculation is based:

Value of F3	1[a]	2	5	10
Duration of study				
Rodent (months)	≥12	≥6 to <12	≥3 to <6	Shorter durations
Dog or monkey (years)	≥7	≥3.5 to <7	≥2 to <3.5	Shorter durations

[a] At least one half-lifetime, or exposure of pregnant animals throughout organogenesis in a reproductive toxicology study.

Some of these conventions reflect outdated and seldom-met ideals rather than the reality; few, if any, "half-lifetime" safety studies of solvents in non-rodents will be found in the literature, and when they are probably they will fall short of modern standards. The longest studies in dogs or nonhuman primates will involve treatment for 9–12 months, and those will be relatively rare. Most investigations of solvent toxicity have been carried out in rodents. Finally, almost any categorical approach suffers from sharp cutoff values that are biologically irrelevant, and a degree of common sense is required at times, for example, dosing for 90 days constitutes a 3-month study even if pedantic opinion holds that the latter should encompass at least 91 days.

The remaining modifying factors are less prescriptive and demand a toxicologist's judgment. F4 varies with the seriousness or severity of the toxicity observed at the effect dose(s) in the animal studies evaluated. Damage to the nervous system, reproductive organs, or critical sensory system organs such as the eyes is regarded as of particular concern. An example of adverse effect quoted in ICH Q3C is non-genotoxic carcinogenesis. In this instance F4 may be taken as 10 by default; more discriminating assessment ideally requires a level of understanding that may be difficult to attain. Are the tumors seen definitely of non-genotoxic origin? Has a sufficiently comprehensive battery of genetic toxicity assays been reported? If the carcinogenic mechanism is hormonal or related to chronic low-grade tissue damage, is it of relevance to humans? Uncertainty can create a dilemma that is not necessarily easily resolved. Generating "missing" genotoxicity assay data is a relatively quick and inexpensive undertaking, and negative results coupled with evidence of carcinogenic potential in animals have been invoked to support an epigenetic mechanism for solvents producing the latter finding. However, potent and multisite tumorigenesis has been associated occasionally with compounds that test negative in some common genotoxicity screens, and careful examination of carcinogenicity study outcomes is advisable. For F4, teratogenicity in reproductive studies is a special case. Fetal malformations without maternal toxicity suggest a direct effect on *in utero* development and warrant a high safety factor (F4 = 10). Malformations seen in the presence of maternal

toxicity, especially if they are recognized as associated with "stress," incur a lower factor (default F4 ≤ 5).

F5, taking values of up to 10, is stated in ICH Q3C as to be used when only an LOEL is available and is modulated by the nature of the toxicity described, although F5 > 1 could also be applied in circumstances where a characteristic of the study or finding is not addressed by the other factors.

Thus, in the appropriate circumstances a denominator as large as 1.2×10^5 to 2×10^4, depending on the species, could be utilized in the equation defining the PDE. In practically all instances factors of this magnitude applied to sound data should be sufficient to reasonably ensure the safety of patients exposed to pharmaceutical products.

In converting mg/kg to mg/day, a body weight of 50 kg is assumed for an adult. While some adults may weigh less than this, the other factors used in determining a PDE are considered to provide adequate cover. Discussion of safety in children led to the inclusion in the guideline of the scope to adjust body weight for a medicine intended solely for pediatric use. Where such adjustment is advisable, it may be most appropriate to choose the weight of the youngest likely patient or the midpoint of standard weights across the pediatric range. Perhaps a little more difficult is the case of medicines intended for both adults and children, although in the author's experience a value provided with reasonable justification is quite likely to be accepted.

The physiological and other constants assumed in calculating solvent intakes (Table 7.4) are based on both published data tables and practical experience. The body weights, in particular, may differ substantially from those used in other reference sources; the body weight of a male SD rat can increase fivefold from 200 g as a young adult to near 1000 g at the end of a 2-year carcinogenicity study. Wherever possible, PDEs are derived from long-term study data, and weights of general toxicology species used are those representing mature animals.

7.5.3.2 Example: *N*,*N*-Dimethylacetamide

The study from which the PDE for *N*,*N*-DMA was derived was carried out by the inhalation route. Use of data from such studies raises the question of whether an adjustment should be made to reflect the fact that typically animals are exposed to the test material for a few hours per day and for fewer than 7 days/week. With rats exposed 6 h/day, 5 days/week for 2 years to an atmosphere containing 100 or 350 ppm, *N*,*N*-DMA showed hepatocellular toxicity and hemosiderin accumulation in Kupffer cells; 25 ppm was an NOEL [5]. Concentration in terms of ppm is converted to mg/l via the equation for an ideal gas, $PV = nRT$, or $n/V = P/RT$; R is taken to be 0.0821 l atm/K/mol and T to be 298 K:

$$25\,\text{ppm} = \frac{25 \times 87.12\left(\text{MW of } N,N-\text{DMA}\right)}{24.45} = 89\,\text{mg/m}^3 = 0.089\,\text{mg/l}$$

Table 7.4 Physiological parameter values used in calculation of PDEs.

Parameter	Species	Value
Body weight	Mouse	28 g
	Mouse (pregnant)	30 g
	Rat	425 g
	Rat (pregnant)	330 g
	Guinea pig	500 g
	Monkey[a]	2.5 kg
	Rabbit (including pregnant)	4 kg
	Dog	11.5 kg
Respiratory volume	Mouse	43 l/day
	Rat	290 l/day
	Guinea pig	430 l/day
	Rabbit	1440 l/day
	Dog	9000 l/day
	Monkey	1150 l/day
	Human	28,800 l/day
Water consumption	Mouse	5 ml/day
	Rat	30 ml/day
Food consumption	Rat	30 g/day

[a] Weight most appropriate to cynomolgus monkey.

and a "correction" made for "continuous dosing":

$$\frac{0.089 \times 6 \times 5}{24 \times 7} = 0.016\,\text{mg/l}$$

There is a sound argument against employment of this "correction," notwithstanding its application in, among other settings, detailed examples of procedures for deriving organic volatile solvents limits proposed by the Pharmaceutical Manufacturers Association in response to USP general chapter <467> (e.g., Pharmacopeial Forum [6]). The multiplier 6/24 attempts to adjust for continuous inhalation of vapor throughout the day, although the 6-h period of exposure delivers a "dose" of the solvent, just as oral administration does, and in the latter case no question of "continuous exposure" arises, even for a compound of short systemic half-life. Nor is there well-founded biological reasoning in the conversion from 5 to 7 days/week administration. Toxic effects of any solvent would be highly unlikely to scale in the linear manner implied on switching from intermittent to uninterrupted exposure, and the latter does not

reflect any realistic therapeutic scenario. The dose adjustment is more plausibly viewed as introducing an additional "safety factor" of 5.6, which when combined with F1 to F5 results in a more conservative PDE:

$$\text{Daily dose} = \frac{0.016 \times 290\,(\text{rat, daily respiratory volume in l})}{0.425} = 10.9\,\text{mg/kg}$$

$$\text{PDE} = \frac{10.9 \times 50}{5\,(\text{F1}) \times 10\,(\text{F2}) \times 1\,(\text{F3}) \times 1\,(\text{F4}) \times 1\,(\text{F5})} = 10.9\,\text{mg/day}$$

F1 = 5, as a study in rats was used to determine the PDE. F2 = 10 to account for variability among individuals. F3, F4, and F5 each = 1, as the 2-year duration of the study effectively covers the animals' lifetime, the toxicity recorded at effect doses was not regarded as severe, and an NOEL was identified in an investigation of acceptable quality.

$$\text{Concentration limit} = \frac{1000 \times 10.9}{10} = 1090\,\text{ppm}$$

7.5.3.3 Example: Toluene

The PDE for toluene is 8.9 mg/day (890 ppm in a product of MDD 10 g/day) and is derived from findings in F344 rats and B6C3F1 mice given up to 5000 mg/kg by oral gavage, 5 days/week in 13-week studies run by the US National Toxicology Program [7]. All animals receiving 5000 mg/kg died during the first week, and deaths also occurred among animals that were given 2500 mg/kg/day. Brain lesions were evident in rats at 1250 mg/kg/day. Increases in liver and kidney weights were regarded as adaptive rather than as evidence of toxicity. A dose of 625 mg/kg/day was concluded to be the NOEL in rats.

Because dosing was carried out on 5 rather than 7 days/week, the first step is to adjust for "continuous dosing," namely,

$$\text{Adjusted dose} = \frac{625 \times 5}{7} = 446\,\text{mg/kg/day}$$

As with *N*,*N*-DMA the rationale for the adjustment is suspect, that is, there is little chance that the dose estimated by such a simple linear conversion would be sustained by a study in which 446 mg/kg actually was administered every day of the week. Again, what this step achieves in practice is to produce a more conservative PDE when the new NOEL is inserted into the relevant calculation:

$$\text{PDE} = \frac{446 \times 50}{5\,(\text{F1}) \times 10\,(\text{F2}) \times 10\,(\text{F3}) \times 5\,(\text{F4}) \times 1\,(\text{F5})} = 8.9\,\text{mg/day}$$

Here, F1 = 5, as the key NOEL was from a study in rats. F2 = 10 to allow for interindividual variation in sensitivity to the effects seen. F3 = 10 based on the duration of the study.[4] F4 = 5 because of the significant nature of the toxicity observed in rats. F5 = 1, as an NOEL, was established, and the study was conducted under the auspices of a well-respected body. Arguably, F4 could have been set at 10, given that brain lesions were reported at higher doses in rats. However, all other evidence considered in establishing the PDE for toluene, including negative carcinogenicity studies from NTP, yielded substantially higher limits, of greater than or equal to 60 mg/day, which would place toluene in class 3. It was considered that the PDE adopted was wholly sufficient to protect patients.

$$\text{Concentration limit} = \frac{1000 \times 8.9}{10} = 890\,\text{ppm}$$

7.5.4 Class 3 Solvents

Degrees of both latitude and subjectivity are apparent in setting PDEs for class 3 solvents. Some are common compounds that have been familiar to the world at large for a very long time indeed and may be in household use, formed naturally in mammalian bodies, generally recognized as safe (GRAS) listed, employed quite freely as food additives, obtainable by the public from stores in very substantial quantities or, in the case of ethanol, be ingested deliberately for social purposes. Perhaps for these reasons, formal and well-conducted toxicity studies are often lacking. Acetic acid is a case in point; it has been taken as vinegar for centuries, in amounts markedly exceeding those likely to be encountered in pharmaceuticals, without significant issues. There has been little impetus to revisit food additive limits set in the 1960s, and a general concordance that irritation and corrosiveness are its foremost adverse effects. Almost all of the animal toxicity studies that are available are of venerable age (1920s, 1940s), and with serious flaws by current standards. Acetic acid is negative in Ames genotoxicity assays, and its PDE of 3200 mg/day is based on an oral fetal development study in rabbits in which 1.6 g/kg was the NOEL. Practically no other information of relevance was located.

Documentation of the calculations underlying the ICH Q3C guideline's PDEs [8] does not give an actual value for anisole, but it is assumed to be more than 50 mg/day based on long and extensive experience, low acute toxicity, GRAS status, and use as a food additive. Isobutyl acetate is in a similar situation. 1-Butanol and isopropyl acetate, for example, at least are negative

4 This reflects an error, or at least an anomaly, in the rationale applied to the calculation underlying the PDE for toluene. According to the ICH Q3C guideline, F3 = 5 for a 3-month (i.e., 13-week) study in rodents. No reason for an exception to this is given in [8]. If F3 is set to 5, the PDE becomes 17.8 mg/day (1780 ppm in a 10 g product).

in genotoxicity testing systems and are also direct food additives, but none of these solvents have been the subject of repeated dose general toxicity studies that allow estimation of a PDE using the fundamental calculation shown earlier.

Should these common and long-used solvents be included in ICH Q3C? If the admission criteria were followed strictly, possibly not, they do not raise suspicion of causing harm and are food additives; it is scarcely rational to tolerate esters and alcohols listed as "no safety concern" for use in foodstuff and then to deny lower intakes via pharmaceutical products. In addition, while the guideline seeks assiduously to maintain patients' safety by setting limits for solvents that have been more comprehensively studied because concern exists, it remains a practical document that does not aim to hamper access to medicines through unduly high costs or impracticality of manufacture.

Ethanol raises a slightly different paradox. Deliberate intake by humans since possibly neolithic times, natural occurrence, and multiple everyday uses provide little incentive for conduct of classical dose–response curve-based toxicity studies. However, the same considerations provoke an interest in ethanol's subtle effects, and not surprisingly these can be detected in humans after ingestion of very small amounts if sufficiently sensitive techniques are employed. Application of merely the safety factors F2 and F5 (an NOEL will generally not be identified because of the nature of these investigations) then gives a PDE implying that ethanol is extraordinarily toxic, and a concentration limit that is probably unachievable by realistic manufacturing practices. Conversely, various countries' health-based recommendations support an intake of at least 80 g/week via alcoholic drinks as being without significant risk. Pragmatism is required.

7.5.5 Solvents for Which Toxicity Data Were Inadequate to Support Derivation of a PDE ("Class 4" Solvents)

As noted elsewhere, the solvents addressed by the ICH Q3C guideline were not identified in a rigorously systematic manner and were certainly not intended or expected to constitute a definitive list of those valuable to the pharmaceutical industry. Ten of the solvents named as of interest proved to have an insufficient safety database from which to determine a PDE or to make a presumption of acceptability founded on their established use as food additives, for example (Table 7.5). This posed the dilemma of whether or not they should be recognized in the guideline at all. Including them would highlight that the industry recognized them as important and might be seen as a call for interested parties to continue searching for substantial data that had been missed, or, rather forlornly, as hoped that new studies might be conducted if the need was great enough. Less usefully, it might also convey the impression that the group, added to the classes 1, 2, and 3 solvents, rounded off a "complete" list. The

Table 7.5 Solvents listed in Q3C(R5) but not assigned a PDE.[a]

1,1-Diethoxypropane	Methylisopropyl ketone
1,1-Dimethoxymethane	Methyltetrahydrofuran
2,2-Dimethoxypropane	Petroleum ether
Isooctane	Trichloroacetic acid
Isopropyl ether	Trifluoroacetic acid

[a] Available safety data determined to be inadequate to support PDE calculation.

latter is reflected to a degree in that, while the classification is not used in ICH Q3C, the compounds in Table 7.5 are referred to by many as "class 4" solvents [9]. In fact, if appropriate information does become available, there is a process in place for proposing PDE values and classifying these and any newly important solvents (see Section 7.7).

7.6 Analysis and Reporting

The foregoing deals principally with the rationale for and derivation of residual solvent limits, their practical application and gaps, quirks and anomalies evident initially or encountered during their use. However, clearly it is necessary to determine compliance practically. Testing for residual solvents should be carried out when production or purification processes are known to be capable of leading to their presence. It is necessary to test only for the solvents used or produced in the manufacture of the drug substance, the product, or the excipients. Furthermore, consideration should be given to factors such as proximity to final product and the characteristics of the manufacturing process. The use of a highly volatile solvent such as diethyl ether at an early stage is very unlikely to present a significant risk in terms of carryover where the process involves multiple subsequent operations such as product crystallization or solvent distillation and drying. This rationale has been justified with respect to other types of impurities, in particular those with mutagenic liabilities [10].

There was some concern at the time of first composition of the ICH Q3C guideline that excipient producers' desire to avoid revealing confidential commercial processes would result in reluctance or refusal to divulge the solvents employed. However, this does not appear to have been a major issue. As described in Section 7.3.1, a cumulative calculation can be applied to estimate residual solvent levels in the drug product where information is available on all components of the formulation. Alternatively, the drug substance or drug product can be analyzed, either routinely or only to determine whether losses during manufacturing or formulation have lowered solvent concentrations to an acceptable level.

The ICH Q3D guideline contains provision for considering the significance of the observed level of an element present in a product relative to the corresponding PDE, with a control threshold at 30% of the PDE [1]. If the concentration of the element from all sources is consistently less than 30% of the PDE and, assuming both appropriate data assessment and adequate control strategy, additional controls are not required. Insofar as a parallel approach is described in relation to residual solvent levels, it is of narrower application than in Q3D. Annexes to the EMA residual solvents guideline [11] provide that when a class 1 solvent might be present in another solvent, a routine test for the former in a suitable intermediate or in the final drug substance is not required if it is demonstrated that not more than 30% of the specified limit is consistently present in six consecutive pilot scale or three consecutive industrial scale batches. Two additional conditions pertaining to the originator solvent must also be met [11].

The same Annexes state that a class 2 solvent used prior to the last stage of synthesis does not have to be included in the specification if it can be shown that its level in the drug substance or an appropriate intermediate is not more than 10% of the acceptable concentration limit defined in the ICH Q3C guideline. Levels of class 2 solvents employed in the final synthetic step should be routinely controlled in the final drug substance. Despite the "10% rule" applied here, in contrast to the "30% rule" of Q3D, in practice it is likely that the former will generally preclude having to test for multiple solvents used in the upstream process.

In considering quality aspects, it is relevant to raise the question of whether manufacturing capability should contribute to the setting of solvent limits, in addition to safety. In particular, if the level of a solvent can be reduced routinely to well below that supported by safety data, should the effective limit be adjusted to reflect manufacturing capability? The answer is a straightforward "no." Control of solvent concentrations to at or below a limit derived systematically from toxicity study outcomes should preclude any need to lower them further. Indeed, were this to be done and batch(es) found to fail the tightened specification, reworking or destruction of material containing demonstrably safe levels of a solvent would in no way serve patients' interests.

Procedures described in the pharmacopoeias can be applied to determine levels of residual solvents. In this respect, the extensive revision of USP general chapter <467> (as *Residual Solvents*) is valuable. Otherwise, manufacturers are free to select the most appropriate validated analytical procedure (validation to conform to relevant current ICH guidelines). Various useful reviews covering current and evolving gas chromatographic techniques, including static and dynamic headspace methods, solid-phase microextraction, single-drop microextraction, and others, have appeared in recent years [12–14]. Where only class 3 solvents may be present, a nonspecific method such as loss on drying can be used.

Examples of statements from producers of drug substances or excipients for drug product manufacture that would constitute evidence of acceptable residual solvent content are provided in the ICH Q3C guideline. Class 1 solvents should invariably be identified and quantified specifically. Likewise, class 2 solvents present at more than their respective option 1 limits, or class 3 solvents present at more than 0.5%, should be identified and quantified.

7.7 Maintenance and Expansion of the ICH Q3C Residual Solvent Guideline

It was agreed by the EWG at the time of adoption of the ICH Q3C guideline that solvent PDEs could and should be updated if additional, appropriate toxicity studies were identified or conducted. This was an important provision to have in place particularly with regard to class 2 solvents where the PDE would benefit from confirmation based on stronger data, or for class 3 solvents that might be "misclassified" on the strength of a borderline package. It was also recognized that new solvents might need to be added to the list and that such action would be welcomed if a data set justifying a PDE were available. A maintenance agreement was instituted in 1999 and a Maintenance EWG was formed.

The solvents initially included in ICH Q3C were largely the result of the joint suggestions of members of the EWG and their colleagues. Assembling the list involved a certain amount of cajoling, which is somewhat surprising given the frequency with which the question "Why is solvent X not in ICH Q3C?" has arisen. In its turn, it may therefore also be seen as curious that relatively little maintenance activity has been requested. Inclusion of a new solvent in the guideline, or acceptance of revision of an existing limit, depends on the performance of the same type of calculation as that used in generating existing guideline values, leveraging quantitatively and qualitatively adequate data. The data and worked estimate of the PDE are submitted to the Maintenance EWG; on its positive review and completion of a period of public consultation, the new limit is incorporated into the document, which is published in its amended form under a revision number. This approach has been used to revise the PDE for tetrahydrofuran (THF) from 121 to 7.2 mg/day, based on the availability of high quality NTP carcinogenicity studies. These showed some carcinogenic potential, but accompanying genotoxicity assays were negative and THF was moved accordingly from class 3 to class 2. Revised limits are subject to the same stepwise process as the original guideline; approval by the ICH Steering Committee of the new PDE for THF under step 4 occurred in September 2002 in the revision of the guideline renamed ICH Q3C(R1).

The PDE for *N*-methylpyrrolidone (NMP) subsequently was on the cusp of being changed from 48.4 mg/day (class 2) to 207 mg/day (class 3) on the strength of a new 2-year feeding study in rats when evaluation of another

investigation, in this case of reproductive toxicity, suggested impairment of pre-weaning development and of higher cognitive function in rat pups whose dams were exposed to 150 ppm NMP at 6 h/day throughout organogenesis. While the nature of the finding did not affect NMP's class 2 status, the PDE was reduced to 5.3 mg/day (step 4 September 2002; ICH Q3C(R2)). This amendment illustrates significant points about the struggle between reliance on the "best" or most appropriate study versus the one giving the lowest PDE, and the vagaries of impact of dose selection. In practice, when multiple data sources are available, typically the PDE for most ICH Q3C-listed solvents has been taken as the lowest PDE generated by analysis of those studies regarded as being of acceptable quality. This may also be the "best" study in terms of dose route, design, duration, provenance, or sheer excellence of conduct (although this last attribute may be very difficult to gauge), but if, say, a 3-month study produces a lower candidate PDE value than an intrinsically more reliable 2-year study, the former limit may be adopted. This is an entirely plausible scenario, depending on the route of administration, scaling of the dose levels selected for the studies, the range of end points measured, the actual nature and severity of the findings, the identification of an NOEL or LOEL, and the species tested in the key study. The initial willingness to *raise* the PDE for NMP considerably based on the appearance of information (the 2-year rat feeding study) judged to be more appropriate than the inhalation carcinogenicity study in rats from which the initial PDE was derived suggests a welcome step forward in evaluation of data source quality and general appropriateness. However, the most applicable study is not always as clear as it appears to be in this instance, and the choice may not be even close to like-for-like. Moving wholesale to use of the "best study" also increases the contentious role of expert opinion (subjectivity), the assessment of *all* studies on a given solvent and defense of the one generating the PDE (resource use), and, for consistency, the reevaluation of the current PDEs. Secondly, the PDE for NMP was lowered before the 207 mg/day limit became effective, as a consequence of a paper reporting the outcome of an inhalation-dosed fetal development study. Interpretation of published results at second hand is not always straightforward; in this case they may or may not indicate selective functional neurotoxicity, which was regarded as the key finding in recalculating the PDE. Nonetheless, the principle of caution won out in this case, with application of a total safety factor of 1250 to the adjusted reproductive study dose level and adoption of a PDE of 5.3 mg/day. ICH Q3C(R3) of November 2005 included both the PDEs for THF and NMP and a correction to the calculation for NMP, although it was only in ICH Q3C(R4) of February 2009 that relevant tables and Appendix 1 were formally updated to contain the revised PDEs for these two solvents.

Amendment of the PDE for cumene (isopropylbenzene) led to the renaming of the guideline as ICH Q3C(R5) in February 2011 and illustrated an important change arising from the advent of undoubtedly more definitive studies, albeit

ones revealing significant toxicity. The original PDE of 55 mg/day (class 3) was assigned on the basis of negative genotoxicity studies and a 6-month rat study suggesting an oral NOEL of 154 mg/kg given 5 days/week with a minimal toxicity of up to 769 mg/kg/day. There had been some discomfort with this study because of its age, the publication date being 1956. An opportunity to revisit the PDE was taken when NTP reported rodent inhalation carcinogenicity studies in 2008 and 2009. The overall findings gave mixed signals requiring toxicologists' judgment, and in this case the most strictly conservative route was not followed. Incidences of respiratory pathway tumors were elevated in rats of both sexes, and of renal tumors in males, even at the lowest concentration of 250 ppm (6 h/day, 5 days/week for 2 years; estimated dose 150 mg/kg/day). Mice showed an increase in airway neoplasms at 125 ppm (estimated dose 169 mg/kg/day) in both sexes, and a dose-related increase in hepatocellular adenoma + carcinoma in females. Bacterial mutagenicity assay results remained of no concern, whereas there was a small but significant increase in micronucleated polychromatic erythrocyte count in rat bone marrow, albeit no corresponding effect in peripheral red blood cells after inhalation of cumene for 3 months. Conversely, the *p53* and *K-ras* mutation pattern in mouse lung tumors suggested DNA damage.

Concern was somewhat lessened by the observation that most tumors were of tissues particularly exposed via the route of administration, that is, inhalation, although of course this mimics the method of use of some pharmaceutical products, and there were neoplastic changes in the liver and the kidney. Some relevant assays indicated a low genotoxic potential, whereas the micronucleus study in rats suggested clastogenicity and mutations were identified within tumors. These seemingly conflicting elements need to be balanced *before* the process of calculating a PDE can be carried out. If cumene is judged on the weight of evidence to be likely to be a genotoxic carcinogen, normal ICH Q3C practice should move it to class 1. If the data set allows sufficient confidence in an alternative mechanism, the solvent may be retained in class 2, although with application of a large composite safety factor. The decision fell in favor of the latter: the lower LOEL in mice was used to derive the PDE, which incorporated the safety factors, F1 = 12 (definitive study in mice), F2 = 10 (human interindividual variation), F3 = 1 (lifetime treatment), F4 = 10 (oncogenic effect detected), and F5 = 10 (no NOEL established). The reasoning provided for F4 = 10 is instructive: the value given to the factor can be rationalized as reflecting the manifestation of severe toxicity in a broad sense, rather than the findings indicating selectively a risk of oncogenicity in humans. If the increased tumor incidence in rodents were to be interpreted as predictive of events in humans, it would raise the question of whether tumors of non-genotoxic origin should always be regarded as less concerning than those triggered by direct interaction with genes. No argument for mechanism or threshold is made in the data analysis appended to ICH Q3C as Part IV. However, faced with comparable

findings from animal carcinogenicity studies, such considerations would be valuable when submitting a case for revision of a solvent PDE.

No revision of the ICH Q3C guideline has been driven by the addition of a solvent in almost 20 years since the original document attained step 4, although the issue of ICH Q3C(R6) for public consultation in July 2015 seems destined to rectify that, as it proposes a PDE for a new solvent, triethylamine (TEA), as well as the revision of the PDE for methylisobutylketone (MIBK, the fourth to be amended). The reasoning applied to revision of MIBK's PDE is very similar to that already provided in the first example in Section 7.1, and the same value, 22.5 mg/day, emerges. Adoption will shift MIBK from class 3 to class 2. A PDE of 62.5 mg/day (6250 ppm, class 3) is proposed for TEA. Essentially, the limit is based on a single inhalation study in rats and evidence of lack of mutagenicity, with no reproductive toxicity data, but the overall package is no more sparse than that accepted as the basis for inclusion of several other solvents in the ICH Q3C guideline. Example calculations based on limited information on another solvent, isopropyl ether, as a candidate for adoption in ICH Q3C are provided in Section 7.1.

If anything, concern about the potential environmental toxicity of certain solvents, most notably dichloromethane, has led to a voluntary and informal tightening of the existing list. Awareness of environmental risk has given rise to interest in so-called "green solvents" such as the ethereal molecules, cyclopentyl methyl ether (CPME) and 2-methyltetrahydrofuran (2-MeTHF). While the ICH Q3C guideline is principally intended to protect patients from the adverse effects of direct delivery of a residual "dose" of solvent, it is entirely legitimate to consider limiting damage to the broader environment, and incidentally those same patients, by use of less toxic solvents if they are appropriate in other respects. Nonclinical studies of CPME have been reviewed according to ICH Q3C principles in at least two papers [15, 16], but unfortunately they raise several practical issues and anomalies. Watanabe [15] appears to eschew a 3-month inhalation study in rats to derive a PDE from an oral study of only 28 days' duration. Despite reference to CPME as a "low toxic alternative solvent," the PDE thereby emerges as 1.5 mg/day, among the ostensibly *most* toxic of class 2 solvents. No workings are provided for the derivation of the PDE. Antonucci et al. [16] mention a 3-month oral toxicity study with CPME, giving almost no detail from which to assess its quality, and suggest a PDE of 7.4 mg/day, as well as an equally unexplained PDE of 6.2 mg/day for 2-MeTHF. Neither paper as it stands would support an adequate case for proposal of a PDE to the ICH Q3C Maintenance EWG nor does a submission appear to have been made. This is regrettable, and it is to be hoped that more effort will be made to both identify and characterize "green solvents" in the near future.

Notwithstanding the relative neglect of the mechanism for extending the number of solvents covered by ICH Q3C, as well as for revising existing limits, it is there to be used when appropriate; the following address its application in more detail.

7.8 Example: Case for Revision of an Existing Residual Solvent Limit

Here the possibility of revision of the limit for MIBK, currently a class 3 solvent, is considered based on the outcome of studies conducted by the US National Toxicology Program and published in the literature in 2008 [17]. NTP rodent carcinogenicity studies are peer reviewed and generally of a quality that lends confidence to risk assessment. MIBK was reported to be negative in a wide range of *in vitro* and *in vivo* genotoxicity studies, including a *Salmonella* reverse mutation assay conducted by NTP [17] (package of assays reviewed by IARC [18]). F344 rats were exposed to MIBK by inhalation for 6 h/day, 5 days/week for 2 years at concentrations of 0, 450, 900, and 1800 ppm. In males, there were slight increases in the incidences of renal tubular adenomas and combined adenomas and carcinomas at 900 and 1800 ppm. Increased incidences of chronic nephropathy were evident in males and females at 1800 ppm, of transitional epithelial hyperplasia of the renal pelvis in males at greater than or equal to 900 ppm, and of mineralization of the renal papilla in males of all exposed groups. Renal tubular hyperplasia occurred in males of all groups of MIBK-exposed rats. The findings were considered to represent a continuum in progression of proliferative lesions of the renal tubular epithelium. B6C3F1 mice exposed to 1800 ppm MIBK under the same conditions showed increased incidences of hepatocellular tumors.

Shorter-term studies indicate that MIBK produces α2μ-globulin nephropathy in male rats, and the role of this male rat-specific phenomenon in renal carcinogenesis is not regarded as relevant to humans. However, a detailed evaluation of the NTP study findings by an IARC committee concluded that the relevance of the tumor response to humans could not be excluded [18]. This accorded with a suggestion by the authors of the 2-year studies that increases in the incidence of at least chronic progressive nephropathy depended on mechanism(s) beyond those mediated by α2μ-globulin.

There is no evidence to suggest that MIBK tumorigenesis in rats was the result of a genotoxic mechanism. An inhaled concentration of 450 ppm was an NOEL for tumors, although it could be regarded only as an LOEL for the continuum of renal effect.

Converting 450 ppm to w/v concentration,

$$450\,\text{ppm} = \frac{450 \times 100.16}{24.45} = 1843\,\text{mg/m}^3 = 1.84\,\text{mg/l}$$

$$\text{For continuous exposure, adjusted dose} = \frac{1.84 \times 6 \times 5}{24 \times 7} = 0.33\,\text{mg/l}$$

$$\text{Daily dose} = \frac{0.33 \times 290}{0.425} = 225\,\text{mg/kg}$$

$$\text{PDE} = \frac{225 \times 50}{5(\text{F1}) \times 10(\text{F2}) \times 1(\text{F3}) \times 2(\text{F4}) \times 5(\text{F5})} = 22.5\,\text{mg/day}$$

$$\text{Concentration limit} = \frac{22.5 \times 1000}{10} = 2250\,\text{ppm}$$

In this case, F1 (rat study), F2 (human variation), and F3 (rodent lifetime study) are quite straightforward. However, F4 and F5 are more open to debate. F4 should be set taking into account that MIBK elicited excess tumors in both rats and mice. Although this apparently occurred via a non-genotoxic mechanism, neither the possibility that the chronic progressive nephropathy seen in rats at 450 ppm is related to tumorigenesis nor potential relevance to humans can be excluded [18]; that is, an argument could be made for a safety factor higher than 2. F5 could be reasoned to be 1, as an NOEL for tumor production, but there was no NOEL for renal tissue changes. Hence, shifting of weight between F4 and F5 might be involved in presenting a case for a revised limit for MIBK to the ICH Q3C Maintenance EWG, but the net outcome would be likely to move this solvent from class 3 to class 2.

7.9 Example: Case for Addition of a Residual Solvent to the ICH Q3C Guideline List

In this example, the possibility of supporting a PDE for isopropyl ether (diisopropyl ether), a solvent originally listed as lacking adequate toxicological data, is considered. The summary of findings on which calculations are based, or studies excluded, is presented very briefly; more details are available from the cited papers.

Isopropyl ether (diisopropyl ether (DIPE), diisopropyl oxide, 2-isopropoxy propane, 2,2′-oxydipropane; CAS No. 108-20-3)

Genotoxicity DIPE was negative in Ames bacterial, yeast, and mammalian cell (mouse lymphoma) assays at acceptable limit concentrations and did not produce chromosome aberrations in primary rat liver cell culture at 1200 μg/ml [19].

Carcinogenicity The carcinogenic potential of DIPE was studied using an experimental protocol that exceeded minimum requirements in some respects but was deficient in other important elements [20]. Groups of 100 male and 100 female SD rats were dosed orally, but on only 4 days/week for 78 weeks rather than 2 years, and then left off dose until spontaneous death, and only two dose levels were employed, 250 and 1000 mg/kg/day. Increases in incidence of several tumor types and sites were reported by the authors at both dose levels. Significant discrepancies, including conclusions apparently

unsupported by the results provided, failure to take into account historical control data, and others, led an analysis by the European Chemicals Agency (ECHA) to conclude that the study was of limited usefulness for identifying the potential carcinogenicity of DIPE [21]. If any of the claimed increases in tumor incidence can be taken as having substance, based on genotoxicity assay results and the precedent of other residual solvents, DIPE would be regarded as a non-genotoxic carcinogen. Application of the standard calculation, including adjustment for dosing 7 days/week, would supply a PDE of 1.4 mg/day, but the ECHA comments on the study render this limit of doubtful reliability, and potentially too stringent.

Reproductive toxicity Pregnant female SD rats were exposed to 0, 430, 3095, or 6745 ppm DIPE for 6 h/day by inhalation throughout organogenesis [22]. There was an increase in incidence of rudimentary or short 14th ribs in fetuses at equal to or greater than 3095 ppm, although the authors stated that "the observed increase … does not appear to be indicative of an adverse effect on development." Mild maternal toxicity was evident at the highest concentration, but no adverse effects on either dams or fetuses at 430 ppm (NOEL).

$$430\,\text{ppm} = \frac{430 \times 102.18}{24.45} = 1797\,\text{mg/m}^3 = 1.8\,\text{mg/l}$$

$$\text{For continuous exposure, adjusted dose} = \frac{1.8 \times 6}{24} = 0.45\,\text{mg/l}$$

$$\text{Daily dose} = \frac{0.45 \times 290}{0.33} = 395\,\text{mg/kg}$$

$$\text{PDE} = \frac{395 \times 50}{5\,(\text{F1}) \times 10\,(\text{F2}) \times 1\,(\text{F3}) \times 1\,(\text{F4}) \times 1\,(\text{F5})} = 395\,\text{mg/day}$$

$$\text{Concentration limit} = \frac{395 \times 1000}{10} = 39{,}500\,\text{ppm}$$

This value is predicated on the authors' interpretation that there was no adverse effect on development, that is, that the observations concerning the 14th rib were essentially an anomaly. A higher level of concern potentially would reduce the PDE tenfold, to 39.5 mg/day (3950 ppm).

Animal Toxicity The oral LD50 in rats was reported to be 6.4–16.5 ml/kg (4.64–12.0 g/kg) [23].

SD rats were exposed to 0, 480, 3300, or 7100 ppm DIPE, 6 h/day, 5 days/week by inhalation for 3 months [21]. Minor increases in liver and kidney weights were apparent at the mid and high concentrations, with slight morphological effects in males at 7100 ppm (hepatocellular hypertrophy and

hyaline droplets in renal tubules; the latter finding is not relevant to humans). The study appears to be of high quality, and a concentration of 480 ppm can be taken as a very conservative NOEL.

$$480\,\text{ppm} = \frac{480 \times 102.18}{24.45} = 2006\,\text{mg/m}^3 = 2.0\,\text{mg/l}$$

$$\text{For continuous exposure, adjusted dose} = \frac{2.0 \times 6 \times 5}{24 \times 7} = 0.36\,\text{mg/l}$$

$$\text{Daily dose} = \frac{0.36 \times 290}{0.425} = 245\,\text{mg/kg}$$

$$\text{PDE} = \frac{245 \times 50}{5(\text{F1}) \times 10(\text{F2}) \times 5(\text{F3}) \times 1(\text{F4}) \times 1(\text{F5})} = 49\,\text{mg/day}$$

$$\text{Concentration limit} = \frac{49 \times 1000}{10} = 4900\,\text{ppm}$$

Conclusion Taking the PDE to be 49 mg/day would place isopropyl ether just inside ICH Q3C class 2.

As is often the case, there is scope for differences of opinion about some of the factors applied and the strength of data available, but in the context of other solvents included in the guideline, sound reasons for excluding the carcinogenicity study outcome, and conservative analysis of the 3-month study in rats, the PDE derived could be proposed as justifiable.

7.10 Appraisal of the ICH Q3C Residual Solvents Guideline 17 Years After Step 4

Considering the paucity of adequate safety data on many solvents, the intrinsic weaknesses of the "simple" uncertainty factor-based approach to limits that is imposed by lack of mechanistic studies, and the subjective judgments inherent in the overall process, the ICH Q3C residual solvents guideline has stood the test of time perhaps somewhat better than expected. The complications of determining individual limits for specified chemical entities have contributed to some anomalies and idiosyncrasies, but the path steered between a generally conservative attitude to ensuring safety, and acceptability to drug product manufacturers, appears largely to have served its purpose.

This is not to say that ICH Q3C is without embedded issues, shortcomings revealed by the march of time, and a degree of stagnation. In certain respects the guideline shows its age. For example, the days of whole- or even

half-lifetime studies in non-rodents are long gone, if they ever really existed. It has to be acknowledged that any long-term study, in life-span terms, will be in rodents, and almost invariably in rats or mice. The thorny and increasingly common issue of polypharmacy is sidestepped and with it potential additive exposure to residual solvents in multiple active ingredients and excipients that may exceed PDEs. However, this is still the rule, not the exception, with guidelines, and the customary justification is that the limits are sufficiently conservative to accommodate most circumstances.

Some of the conventions and accommodations applied to elements of ICH Q3C probably warrant reexamination. Class 1 solvents have been the target of some comment because of the use of concentrations, and not PDEs, to define their limits in pharmaceutical products, and the limit for benzene in particular has been criticized as unnecessarily low. As noted in 4.1, the absence of PDEs for class 1 solvents result in certain anomalies and, notwithstanding desire for reinforcement of the message that these solvents should be avoided during manufacture, the concentrations themselves are derived from PDE calculations. The ICH Q3C concentration limit for benzene, 2 ppm, reflects a PDE of 0.02 mg/day, which is based on a lifetime excess cancer (leukemia) risk of 10^{-5}. This EPA estimate has been updated to give only a range of exposures [24]. Snodin and McCrossen [25] considered World Health Organization (WHO), Agency for Toxic Substances and Disease Registry (ATSDR), and Carcinogenic Potency Database (CPDB) sources, which give limits of 20–78 μg/day (10^{-5} excess cancer risk), and environmental exposures of several hundred micrograms, to argue that the ICH Q3C limit is excessively conservative. They proposed additionally that more flexibility and consistency would be achieved if a PDE (i.e., dose) instead of concentration was employed for benzene, even if that remained as currently implied, at 0.02 mg/day (20 μg/day).

The unsophisticated nature (in risk assessment terms) of the safety or uncertainty factor approach begs the question of whether alternative means could be used to generate PDEs, where sufficient variety and reliability of information are available. Most often this will not be the situation, but interest in a solvent such as dichloromethane for environmental reasons has led to in-depth investigation of its toxic and carcinogenic potential, and mechanistic risk assessments based on a PBPK approach [26]. If this, or other, type of calculation enhanced the rationale underlying a PDE, but raised the limit, should it be acceptable? Would consistency of method be preferred over improved evaluation of risk? In addition, guidance to the more rigorous use of the "best" (which may often be interpreted as the longest) study or the one generating the lowest PDE value might be welcomed. These studies may prove to be the same ones, insofar as prolonged dosing tends to be associated with lowering of the NOEL, but it is far from assured, and PDEs inevitably have substantial dependence on study design, especially on the range and separation of the dose levels chosen. Related to this is the question of whether PDEs should be estimated from the

NOEL in investigations of teratogenic potential, which may be the case if few general toxicology studies are available, or possibly because the lowest candidate PDE calculated arises from such an investigation. There is an argument that evidence of ability to cause fetal malformations should influence the safety factor (F4), but that the NOEL obtained in this specialized type of assessment is not the most appropriate one on which to base the overall PDE. Should route of administration be taken into account, as in Q3D, and multiple PDEs generated for each solvent? Certainly there is a theoretical rationale for doing so, but the reality is that there would be more gaps than substance, particularly affecting the most important, oral, route, unless one resorted to contrived solutions that would offer little or no improvement of the present situation.

Conflict with other impurity guidance is not unlikely, either now or as new data emerge. ICH guidelines have not evolved entirely in isolation, but it is impossible to make all processes and applications fully compatible. If a residual solvent is not listed in ICH Q3C, an alternative, although less than an ideal one, is to consider it in the context of ICH's general impurity guidelines. When and if a new PDE is added to ICH Q3C it may differ markedly from the previous limit imposed by ICH Q3A [27] or ICH Q3B [28]. Another possibility is that new information will suggest that a solvent is genotoxic and raise the question of whether it should be limited by ICH Q3C class 1 parameters, or under the terms of the ICH M7 guideline [29]. To date, serious conflicts have not arisen, but they may yet occur, and the consequences of their doing so have been the subject of much speculation.

Perhaps the most disappointing aspect of ICH Q3C is the limited updating that has occurred during its lifetime. The original list of solvents was scarcely drawn up systematically, and it was anticipated that there would be an ongoing need to add to it. New studies, while not expected in profusion, would be published and drive revisions to existing PDEs. The PDEs for THF, NMP, and cumene have been amended based on additional data, and revision of the limit for MIBK has been proposed. Metabolic arguments were offered to support upward revision of the PDE for ethylene glycol but rejected by the Maintenance EWG [30]. A case for adding TEA to the guideline has been submitted. To the author's knowledge, this is the extent of maintenance activity. Of relevance, interest in "green solvents" is increasing, and a few examples of their assessment using ICH Q3C principles have been reported [15, 16]. However, the information, or its presentation, so far appears to have fallen short of being sufficient to submit to the Maintenance EWG. The publications also illustrate a need to balance broad environmental benefits and individual patient safety; available information may support the former, while driving a PDE that suggests greater risk to the patient than is presented by most solvents already listed in ICH Q3C. This probably reflects the current dearth of adequate studies rather than intrinsically higher toxicity, and "greener" solvents can be anticipated to be added to the guideline as more work is carried out to characterize them.

Perhaps the industry has been too relieved to have specific guidance to permitted residual levels of most commonly used solvents to wish to tempt fate. There have been criticisms of both principle and detail since 1997, but also appreciation [31], and an observation that the guideline's approach could be adopted more widely with advantage. Nonetheless, neither processes nor actual PDEs should be allowed to stagnate. New methods of risk assessment as well as new data may be used to challenge the status quo, to the longer-term benefit and continued relevance of the guideline.

References

1 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Guideline for Elemental Impurities Q3D. *Step 2b*, July 26, 2013.

2 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Impurities: Guideline for Residual Solvents ICH Q3C(R5). *Step 4*, February 4, 2011.

3 Gaylor D.W., Kodell R.L. (1980) Linear interpolation algorithm for low dose risk assessment of toxic substances. *Journal of Environmental Pathology and Toxicology*, 4, 305–312.

4 US Food and Drug Administration. Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. Center for Drug Evaluation and Research, July 2005.

5 Malley L.A., Slone T.W. Jr, Makovec G.T., Elliott G.S., Kennedy G.L. Jr. (1995) Chronic toxicity/oncogenicity of dimethylacetamide in rats and mice following inhalation exposure. *Fundamental and Applied Toxicology*, 28, 80–93.

6 Committee on Rational Specifications for Impurities in Bulk Drug Substances—Pharmaceutical Manufacturer Association. (1990) Application of the PMA Procedure for Setting Residue Limits for Organic Volatile Solvents in Pharmaceuticals to Chloroform. *Pharmacopeial Forum, May–June,* 541–582.

7 National Toxicology Program. NTP Toxicology and Carcinogenesis Studies of Toluene (CAS No.108-88-3) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). National Toxicology Program Technical Report Series. 371, 1–253, 1990.

8 Connelly J.C., Hasegawa R., McArdle J.V., Tucker M.L. (1997) ICH guideline: residual solvents. *Pharmeuropa (Supplement)*, 9, S1–S68.

9 Grodowska K., Parczewski A. (2010) Organic solvents in the pharmaceutical industry. *Acta Poloniae Pharmaceutica—Drug Research*, 67, 3–12.

10 Teasdale A., Elder D., Chang S-J., Wang S., Thompson R., Benz N., Sanchez Flores. (2013) Risk assessment of genotoxic impurities in new chemical entities: strategies to demonstrate control. *Organic Process Research & Development*, 17, 221–230.

11 European Medicines Agency. Annexes to CPMP/ICH/283/95 Impurities: Guideline for residual solvents & CVMP/VICH/502/99 Guideline on impurities: residual solvents. Annex I: specifications for class 1 and class 2 residual solvents in active substances. Annex II: residues of solvents used in the manufacture of finished products. CPMP/QWP/450/03—Rev. 1, EMEA/CVMP/511/03—Rev. 1. February 20, 2013.

12 Grodowska K., Parczewski A. (2010) Analytical methods for residual solvents determination in pharmaceutical products. *Acta Poloniae Pharmaceutica—Drug Research*, 67, 13–26.

13 Hu C., Liu Y. (2011) Quality control in pharmaceuticals: residual solvents testing and analysis. In Akyar Y. Wide spectra of quality control, chapter 11, Rijeka, Croatia: Intech, pp. 1–29.

14 Kapil M., Lata S. (2013) A review: residual solvents and various effective gas chromatographic techniques in the analysis of residual solvent. *International Journal of Pharma Research & Review*, 2, 25–40.

15 Watanabe K. (2013) The toxicological assessment of cyclopentyl methyl ether (CPME) as a green solvent. *Molecules*, 18, 3183–3294.

16 Antonucci V., Coleman J., Ferry J.B., Johnson N., Mathe M., Scott J.P., Xu J. (2011) Toxicological assessment of 2-methyltetrahydrofuran and cyclopentyl methyl ether in support of their use in pharmaceutical chemical process development. *Organic Process Research & Development*, 15, 939–941.

17 Stout M.D., Herbert R.A., Kissling G.E., Suarez F., Roycroft J.H., Chhabra R.S., Bucher J.R. (2008) Toxicity and carcinogenicity of methyl isobutyl ketone in F344N rats and B6C3F1 mice following 2-year inhalation exposure. *Toxicology*, 244, 209–219.

18 International Agency for Research on Cancer. Monographs on the Evaluation of Carcinogenic Risks to Humans 101. IARC/WHO, 2012.

19 Brooks T.M., Meyer A.L., Hutson D.H. (1988) The genetic toxicology of some hydrocarbon and oxygenated solvents. *Mutagenesis*, 3, 227–232.

20 Belpoggi F., Soffritti M., Minardi F., Bua L., Cattin E., Maltoni C. (2002) Results of long-term carcinogenicity bioassays on tert-amyl-methyl-ether (TAME) and di-isopropyl-ether (DIPE) in rats. *Annals of the New York Academy of Sciences*, 982, 70–86.

21 European Chemicals Agency. http://apps.echa.europa.eu/registered/data/dossiers/DISS-9d9a4cf8-17d0-2fad-e044-00144f67d249/DISS-9d9a4cf8-17d0-2fad-e044-00144f67d249_DISS-9d9a4cf8-17d0-2fad-e044-00144f67d249.html (accessed October 5, 2014).

22 Dalbey W., Feuston M. (1996) Subchronic and developmental toxicity studies of vaporized diisopropyl ether in rats. *Journal of Toxicology and Environmental Health*, 49, 29–43.

23 Kimura E.T., Ebert D.M., Dodge P.W. (1971) Acute toxicity and limits of solvent residue for sixteen organic solvents. *Toxicology and Applied Pharmacology*, 19, 699–704.

24 EPA: Integrated Risk Information System. Benzene http://www.epa.gov/iris/subst/0276.htm (accessed October 23, 2014).
25 Snodin D.J., McCrossen S.D. (2012) Guidelines and pharmacopoeial standards for pharmaceutical impurities: overview and critical assessment. *Regulatory Toxicology and Pharmacology*, 63, 298–312.
26 Marino D.J., Clewell H.J., Gentry P.R., Covington T.R., Hack C.E., David R.M., Morgott D.A. (2006) Revised assessment of cancer risk to dichloromethane: part I Bayesian PBPK and dose–response modeling in mice. *Regulatory Toxicology and Pharmacology*, 45, 44–54.
27 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human use. Impurities: Impurities in New Drug Substances Q3A(R2). *Step 4*, October 25, 2006.
28 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human use. Impurities: Impurities in New Drug Products Q3B(R2). *Step 4*, June 2, 2006.
29 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human use. Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk (M7). *Step 4*, June 23, 2014.
30 Hasegawa R., Koizumi M., Hirose A. (2004) Principles of risk assessment for determining the safety of chemicals: recent assessment of residual solvents in drugs and di(2-ethylhexyl)phthalate. *Congenital Anomalies*, 44, 51–59.
31 Rousseau G., Jeffs P. (2000) International Conference on Harmonization Impurity Guidelines—the industry perspective. *Drug Information Journal*, 34, 903–907.

8

ICH Q3D Elemental Impurities

Andrew Teasdale and Sarah Thompson

AstraZeneca, Macclesfield, UK

8.1 Introduction

The potential presence of metallic impurities within pharmaceutical products is a long-standing concern due to obvious safety hazards. Historically these concerns, focused on heavy metals such as lead, have been addressed through the use of simple "wet chemical" tests. Such tests generally involve the use of hydrogen sulfide, the presence of metals being determined through the observation of a precipitate, and the levels of metals present being visually assessed through comparison with lead standard solutions. At present there are a number of variations of this test, across the main pharmacopoeias:

- USP—Currently 3 variants of the test are described under chapter <231>, "Heavy Metals."
- Ph. Eur.—chapter 2.4.8 of the European Pharmacopoeia contains eight variants.
- JP—Defined in chapter 1.07, this contains four wet chemistry methods.

These variants relate mainly to sample preparation. The test however contains a number of flaws; these include:

- Lack of specificity—a number of metals will form insoluble sulfides, meaning that the level of individual metals cannot be assessed.
- Conversely several metals of importance do not form precipitates and hence may well be missed by the test.

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- Recovery—some of the metals that do form precipitates are underestimated by the test. Volatile elements such as mercury, may be lost during sample preparation.
- Sensitivity is also a concern, especially at the level defined by newly established permitted daily exposure limits as expressed in ICH Q3D [1].

Given such concerns and the now widespread availability of more specific and sensitive instrumental techniques such as ICP-OES, ICP-MS, and XRF, industry and regulators alike saw a need to address this.

In 1995 USP issued a stimuli article [2]; this focused on specific issues with the heavy metal limit test <231>. A second stimuli article [3] proposed the use of ICP-MS as an alternative, with a third article in 2004 [4] for the first time openly proposing the replacement of the heavy metal limit test.

In parallel to the work of the USP, the EMEA (now EMA) developed a guideline specifically focused on metal catalysts [5]. The guideline was finalized and implemented in 2008. This guideline for the first time established formal limits for specific, individual metals, splitting metals into three categories:

Class 1 metals: Metals of significant safety concern. This group includes metals that are known or suspected human carcinogens or possible causative agents of other significant toxicity.

Class 2 metals: Metals of low safety concern. This group includes metals with lower toxic potential to man. They are generally well tolerated up to exposures that are typically encountered with administration of medicinal products. They may be trace metals required for nutritional purposes or they are often present in food stuffs or readily available nutritional supplements.

Class 3 metals: Metals of minimal safety concern. This group includes metals with no significant toxicity. Their safety profile is well established. They are generally well tolerated up to doses that are well beyond those typically encountered with the administration of medicinal products. Typically they are ubiquitous in the environment or the plant and animal kingdoms.

Interestingly the guideline makes no specific recommendation as to the analytical approach beyond indicating that in most circumstances the use of nonspecific precipitation tests was unsuitable and that generally an element-specific technique should be used.

Concurrently USP continued to develop its own principles relating to elemental impurities, publishing this in 2008 within PF 34(5) (Sept.–Oct. 2008)—General Chapter on Inorganic Impurities: Heavy Metals [6]. This listed 31 elements of potential concern and included proposed limits, both oral and parenteral for each element. This went significantly further than the EMA guideline articulating the need for a holistic assessment of the risk posed by metals, moving away from the EMA guideline's specific focus on metal catalysts to a wider focus on all potential sources of metal impurities.

Clearly there was a need to address the divergence in approach, both in terms of the effective scope of guidelines and more specifically the elements of concern and their permitted limits. This led in 2009 to the establishment of an ICH concept paper [7] and the subsequent formation of a formal ICH expert working group (EWG) to develop a new ICH guideline, ICH Q3D Guideline for Elemental Impurities. It is beyond the scope of this chapter to describe in detail the specific challenges faced in the development of the guideline itself, suffice to say that the challenges were significant, resulting in the guideline taking 5 years to finalize. The guideline being formally signed off by the ICH parties in December 2014 [1]. In addition to the guideline itself, the ICH EWG took the unprecedented step immediately after finalization of establishing an implementation working group (IWG); the primary task of which was to produce training materials to support the practical implementation of the guideline. These were published in April 2016 [8] and are examined in the following text.

At the present time there remain challenges regarding practical implementation of the guideline, and it is these challenges that the chapter will primarily focus on.

8.2 Guideline Structure

The guideline totals some 73 pages; however the main body of the guideline, including references and the glossary, is contained within the first 17 pages. The rest of the guideline takes the form of a series of appendices:

- Appendix 1: Method for Establishing Exposure Limits
- Appendix 2: Established Permitted Daily Exposures (PDEs) for Elemental Impurities by Oral, Parenteral, and Inhalation Routes of Administration
- Appendix 3: Individual Safety Assessments for 24 Elements
- Appendix 4: Illustrative Examples

8.3 Scope

ICH Q3D applies to marketed products and is therefore not applicable during clinical development. Like all other similar ICH guidelines, it is to be anticipated that the principles outlined in the guideline will be applied during the clinical stages of development and that a full evaluation of risk and associated control strategy will be established, prior to marketing application. Importantly the guideline makes clear that application to new marketed products was not expected until 18 months after publication and 36 months for existing products. This recognizes the significant challenges in implementation, including the effort required to generate the often necessary data needed to conduct a thorough risk assessment.

The guideline is applicable to both synthetically derived drug products (small molecules) and biologics, reflecting the fact that the guideline relates to the holistic assessment of potential sources of elemental impurities and many of these, for example, water/process equipment, are common to both synthetic and biologic drug products. Gene- and cell-based therapies are however excluded, as are herbal products, radiopharmaceuticals, vaccines, cell metabolites, DNA products, whole blood, and a range of blood derivatives.

Although not clearly stated in the guideline, it is believed that the limits defined are applicable irrespective of the patient population, that is, there is no need for further modification for pediatric populations due to the conservative approach taken in establishment of the PDEs within the guideline.

8.4 Safety Assessment of Potential Elemental Impurities

This section of the guideline (section 3) specifically addresses how limits were derived, outlining the basic high level principles upon which the overall process was based, cross-referencing appendix 1 of the guideline. A detailed overview of the methodology employed, including the selection of modifying factors used in the calculation, is provided. Limits within the guideline are defined in terms of PDEs, this aligning with the process used to establish limits for residual solvents defined in ICH Q3C [9].

The PDE is derived from the no-observed-adverse-effect level (NOAEL) or the lowest-observed-adverse-effect level (LOAEL) in the most relevant animal study as follows:

$$\text{PDE} = \frac{\text{NO(A)EL} \times \text{mass adjustment}}{F1 \times F2 \times F3 \times F4 \times F5}$$

The PDE is derived preferably from a NOAEL. If no NOAEL is obtained, the LOAEL may be used. The modifying factors proposed, for relating the data to humans, are as follows:

$F1$ = A factor to account for extrapolation between species, taking into account comparative surface area, for example, $F1 = 5$ for extrapolation from rats to humans.
$F2$ = A factor of 10 to account for variability between individuals. A factor of 10 is generally given for all elemental impurities, and 10 is used consistently in the guideline.
$F3$ = A variable factor to account for toxicity studies of short-term exposure.
$F4$ = A factor that relates to severity of observed toxicity.
$F5$ = A variable factor that may be applied if the NOEL was not established. For example, $F5 = 1$ for a NOEL and $F5 = 10$ for a LOAEL.

Table 8.1 Permitted daily exposures for elemental impurities.

Element	Class	Oral PDE (μg/day)	Parenteral PDE (μg/day)	Inhalation PDE (μg/day)
Cd	1	5	2	2
Pb	1	5	5	5
As	1	15	15	2
Hg	1	30	3	1
Co	2A	50	5	3
V	2A	100	10	1
Ni	2A	200	20	5
Tl	2B	8	8	8
Au	2B	100	100	1
Pd	2B	100	10	1
Ir	2B	100	10	1
Os	2B	100	10	1
Rh	2B	100	10	1
Ru	2B	100	10	1
Se	2B	150	80	130
Ag	2B	150	10	7
Pt	2B	100	10	1
Li	3	550	250	25
Sb	3	1,200	90	20
Ba	3	1,400	700	300
Mo	3	3,000	1500	10
Cu	3	3,000	300	30
Sn	3	6,000	600	60
Cr	3	11,000	1100	3

The mass adjustment assumes an arbitrary adult human body mass for either sex of 50 kg.

Using this approach limits were established for 24 elements in total; the individual assessments are described in detail in appendix 3 of the guideline. Appendix 2 describes the actual individual limits that are provided for oral, parenteral, and inhalation routes of administration. These are reproduced and shown in Table 8.1.

Some guidance is also provided in terms of principles that may be adopted when seeking to establish limits for other routes of administration.

The starting point advocated by the guideline is to base such limits on the oral PDEs provided in the guideline. Teasdale et al. [10] published a detailed evaluation of the existing data relating to dermal exposure to elemental impurities.

This highlighted the fact that the skin provides a formidable barrier for elemental impurities. Within the skin, the outer layer, the stratum corneum, provides the most significant barrier. The stratum corneum is highly lipophilic with a very low water content and is rate limiting for any substance applied to the skin whether intentional in the form of an ointment or a contaminant inadvertently present on the skin, for example, a metal impurity present in the applied ointment. Penetration of hydrophilic or charged molecules is particularly difficult; such species are effectively unable to partition into the lipid layer and hence pass through the skin at significant level. Although fragmentary in nature, the available data are nevertheless compelling, showing that dermal exposure to metals is limited, often well below 10% of that observed when administered orally. This is entirely consistent with the fundamental nature of the dermis and the obvious barrier it presents to charged materials (such as metal ions) in particular. Reflecting on the variable approaches taken by regulatory bodies, it is clear that even the most conservative of the variable approaches [11] differ substantially from assumed parity with parenteral or even oral exposure. Certainly the assumptions prescribed by Michigan Department of Environmental Quality (MDEQ) and West Virginia Department of Environmental Protection (WVDEP) [12] of exposure levels equivalent to <5% absorption correlate well with the available data. Although the guideline suggests that an assessment of other routes should start with oral limits, it would seem more appropriate to evaluate such risks on a case-by-case basis. It also impacts which metals need to be considered during such an assessment i.e. which classes; based on data, a focus on classes 1 and 2a metals would seem realistic. It is understood at the timing of writing that the IWG plan to examine this further.

8.4.1 Justification for Elemental Impurity Levels Higher Than an Established PDE

The guideline permits the establishment of limits > PDE under specific circumstances; however unlike the duration-based limits defined in ICH M7 [13] applied to mutagenic impurities, based on Haber's law, there is no systematic methodology presented, instead the following situations are described:

- Intermittent dosing
- Short-term dosing (30 days or less)
- Specific indications (e.g., life-threatening, unmet medical needs, rare diseases)

The guideline provides examples of justifying an increased level of an elemental impurity using a sub-factor approach. This involves modification of factor *F*2 (default = 10). This can be subdivided into two sub-factors, one for toxicokinetics (TK) and one for toxicodynamics (TD), each with a range from

1 to 3.16. In practice this may prove challenging as identifying the necessary data, that is, plasma half lives for the element concerned, may be difficult. It would seem prudent to recommend discussing with regulators at an appropriate juncture, for example, end of phase 2 briefing package.

8.5 Element Classification

Elemental impurities are classified based on two factors—toxicity and natural abundance—and placed into three classes. The guideline also acknowledges the existence of a range of nonclassified, nontoxic, elements; these include Al, B, Ca, Fe, K, Mg, Mn, Na, W, and Zn. There are clear differences between this approach and the EMA approach described earlier in the introduction, not only in terms of differences in the list of elements of concern but also in terms of classification criteria. While industry welcomes global harmonization of PDEs and classification systems, withdrawal of the EMA guidance (once ICH Q3D reaches step 5—adoption within the European Region) also represents a significant change and will require care not to confuse the issue.

The three ICH Q3D classes are as follows:

Class 1—There are four elements defined within this class: arsenic (As), mercury (Hg), cadmium (Cd), and lead (Pb). These are defined based on their toxicity, lack of general use in manufacturing processes, and high natural abundance. Specific reference is made to the potential risk of presence in mined excipients, a point evaluated further in the section within this chapter addressing risk assessment, section 8.6. What is required in terms of assessing the risk of the "big 4" is often misinterpreted—the guideline states that the risk posed by the four elements must be *assessed* irrespective of route of administration. Critically though it does not state that this must involve routine analytical testing. Testing is only required, as with any other element, where the risk assessment specifically identifies a need to control/monitor levels to ensure that PDE limits are not exceeded.

Class 2—This is further divided into 2 further subclasses based on natural abundance:

- Class 2A—This includes those elements considered to present a risk based on their relatively high natural abundances; the elements concerned are cobalt (Co), nickel (Ni), and vanadium (V).
- Class 2B—This relates to elements that are generally considered to be of low natural abundance. Such elements need only be considered where deliberately added to the manufacturing process typically as a catalyst. The elements concerned are silver (Ag), gold (Au), iridium (Ir), osmium (Os), palladium (Pd), platinum (Pt), rhodium (Rh), ruthenium (Ru), selenium (Se), and thallium (Tl). A number of these have very specific and therefore limited applications, for example, thallium.

Table 8.2 Elements to be considered in the risk assessment.

Element	Class	If intentionally added (all routes)	If not intentionally added		
			Oral	Parenteral	Inhalation
Cd	1	Yes	Yes	Yes	Yes
Pb	1	Yes	Yes	Yes	Yes
As	1	Yes	Yes	Yes	Yes
Hg	1	Yes	Yes	Yes	Yes
Co	2A	Yes	Yes	Yes	Yes
V	2A	Yes	Yes	Yes	Yes
Ni	2A	Yes	Yes	Yes	Yes
Tl	2B	Yes	No	No	No
Au	2B	Yes	No	No	No
Pd	2B	Yes	No	No	No
Ir	2B	Yes	No	No	No
Os	2B	Yes	No	No	No
Rh	2B	Yes	No	No	No
Ru	2B	Yes	No	No	No
Se	2B	Yes	No	No	No
Ag	2B	Yes	No	No	No
Pt	2B	Yes	No	No	No
Li	3	Yes	No	Yes	Yes
Sb	3	Yes	No	Yes	Yes
Ba	3	Yes	No	No	Yes
Mo	3	Yes	No	No	Yes
Cu	3	Yes	No	Yes	Yes
Sn	3	Yes	No	No	Yes
Cr	3	Yes	No	No	Yes

Class 3—Elements within this class are considered to be of low oral toxicity. Hence risk assessment is only required when potentially present in products intended for inhalation/parenteral administration. Elements included within this class are barium (Ba), chromium (Cr), copper (Cu), lithium (Li), molybdenum (Mo), antimony (Sb), and tin (Sn). It is important to realize that the guideline (see Table 8.2) provides further granularity in that while all should be screened for inhaled products, only lithium, antimony, and copper need to be screened for parenteral drug products.

In earlier drafts of the guideline, a further class, class 4, was defined that sought to encapsulate elements considered to be of low toxicity and/or subject to regional regulations. This classification was removed in the finalized guideline. Its removal undoubtedly presents some challenges, particularly in relation to metals such as aluminum that are subjected to regional guidelines; the absence of definitive global limits made even more challenging by the lack of clarity/visibility of regional guidelines. A specific issue in the United States was preexistence of nutritional guidance relating to permitted levels of Al [14].

This states that the aluminum content of large volume parenteral (LVP) drug products used in total parenteral nutrition (TPN) therapy must not exceed 25 micrograms per liter (μg/l).

The classification system described in the guideline summarizes which elements need to be considered in a risk assessment; see Table 8.2. Elements of low natural abundance need only be considered if intentionally added to the synthetic process, and elements of low oral toxicity need not be considered for oral drug products.

8.6 Risk Assessment

8.6.1 General Principles

Without a doubt the most challenging aspect of the practical implementation of ICH Q3D is the evaluation of risk. The guideline makes clear that this should be based on the principles outlined in ICH Q9 [15] (Quality Risk Management); moreover this should be based on process understanding, aligned to the principles of ICH Q8 [16] (drug product) and ICH Q11 [17] (drug substance). Ultimately the purpose is to assess the risk with respect to the final drug product; however the guideline makes clear the need for a holistic risk assessment, one taking into account all potential sources of elemental impurities. The guideline does not however define how such an assessment should be performed nor what tools should be utilized. The theoretical example shown later in this chapter is based on the use of failure mode, effects, and criticality analysis (FMECA), a well-established formal risk assessment tool; however the guideline also allows other approaches including informal- and company-specific procedures provided their use can be justified. The guideline does however seek to define a threshold for risk, this being <30% of the PDE for the element in question. It is ultimately up to an applicant to define and justify their approach. One concern given the absence of a fully defined framework is that it may well lead, in many cases, to highly risk-averse approaches predicated on substantive screening. The challenge for industry and regulators alike is to ensure that risk assessments remain true to their title, that is, that they properly reflect the level of risk and do not become a template for exhaustive testing.

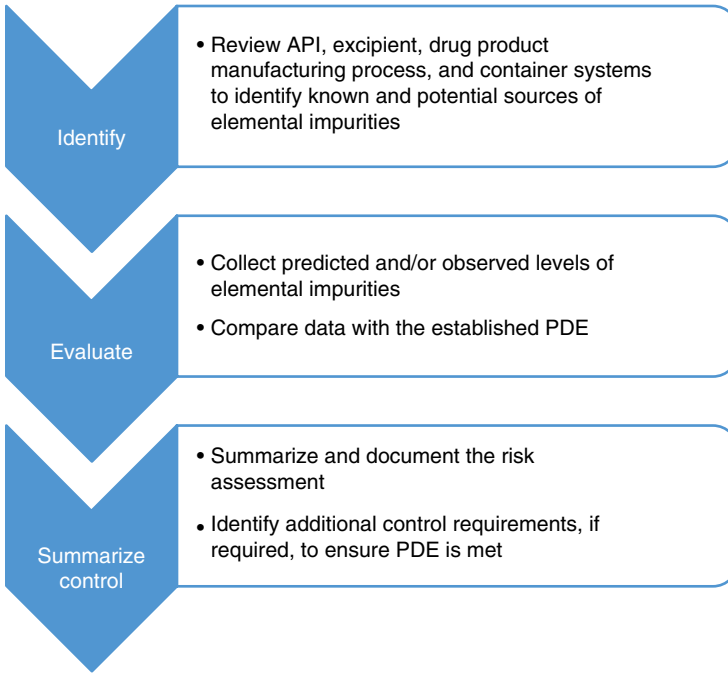

Identify
• Review API, excipient, drug product manufacturing process, and container systems to identify known and potential sources of elemental impurities
Evaluate
• Collect predicted and/or observed levels of elemental impurities
• Compare data with the established PDE
Summarize control
• Summarize and document the risk assessment
• Identify additional control requirements, if required, to ensure PDE is met

Figure 8.1 ICH Q3D risk assessment process.

In terms of the overall assessment process, this is essentially a three-step process as illustrated in Figure 8.1.

It is important to bear in mind this is not always a linear process as many of the specific risk sources discussed in the following text illustrate. This process is often an iterative process that may involve repeated reiterations of some of the steps as knowledge and understanding are continuously developed.

Although applicable only at the marketing phase, it is very likely that a fit-for-purpose assessment of risk will be made during clinical development based on the principles of the guidelines. In phases I/II an initial high level approach, focused on high risk sources, for example, metal catalysts, is typically used. This then evolves through development to a comprehensive assessment, one that underpins the final proposed commercial control strategy.

Each of these steps are examined:

8.6.2 Identify

8.6.2.1 Identify Potential Sources of Elemental Impurities

The guideline provides a useful framework to use for conducting the initial stage of the risk assessment; this is illustrated in the fishbone diagram shown in Figure 8.2.

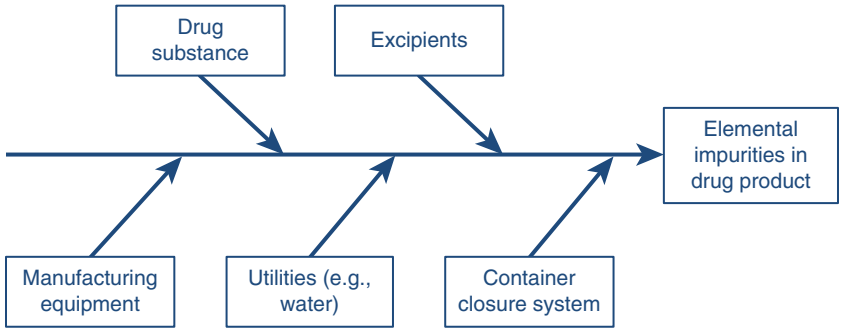


Figure 8.2 Potential sources of elemental impurities in finished drug products.

8.6.3 Identify Potential Elemental Impurities

The first step in the process is to examine each aspect of the process and within this, evaluate what, in reality, are the potential elemental impurities concerned. Wherever possible this should be based on process knowledge, gained directly during development and/or through evaluation of published data, supplier information, and other relevant data.

8.6.3.1 Drug Substance

In respect of the drug substance, arguably the first question to address is where in the process the assessment should start. Manufacturing processes for some drug substances are long/complex syntheses; it is not unusual for some syntheses to exceed 20 chemical steps. Such a decision needs to be addressed in a case-by-case basis; however in general it would seem reasonable to instigate the risk assessment from either the registered starting material (RSM) or from late stages (2 to 3 steps) from the API. In general sources of elemental impurities such as metal catalysts are likely to have a minimal impact where used four or more steps from the API. Most catalysts are heterogeneous in nature, for example 5% Pd on charcoal and in these cases purging will be by isolation/filtration, at the point of use. Should any trace of metal remain in a solubilized form, further purging is likely to occur in the downstream process. Once the start point is established, the next stage is to consider components associated with the manufacturing process that may contribute to the overall EI risk. Figure 8.3 illustrates the primary potential sources of EIs associated with the manufacture of the drug substance.

8.6.3.1.1 Metal Catalysts

The most likely source of EIs in drug substance is the use of intentionally added metals in the form of a metal catalyst. Obviously in such instances the nature of the risk is clear and transparent, that is, the metal in question. When assessing the extent of the risk, key factors for consideration include catalyst loading, as well as the nature of the catalyst used in the process (i.e., homogeneous vs.

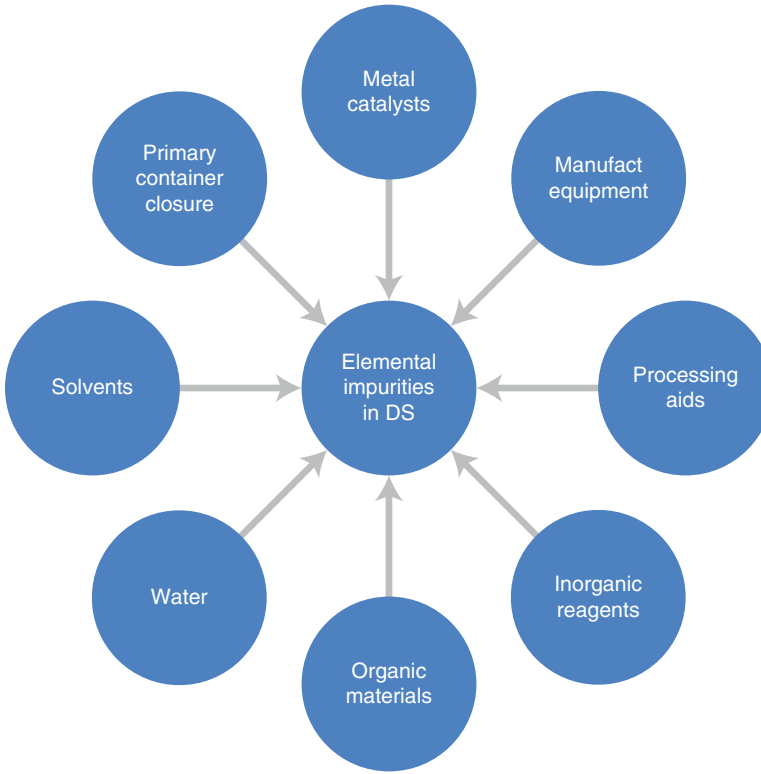


Figure 8.3 Primary potential sources of EIs in drug substance (DS).

heterogeneous catalysts). Heterogeneous catalysts, such as palladium on carbon, are often easily removed from reaction mixtures by filtration, and therefore, the risk of carry-over of elemental impurities into the drug substance is typically low. The advent of the EMA guideline [5] implemented in 2008 has meant that many organizations have started to build up a significant level of knowledge in terms of catalyst levels in drug substance, including an understanding of purging under typical process conditions.

One area of potential concern is complexation/chelation; some APIs are effective chelators, for example, mycophenolic acid forms a complex with iron when in contact with stainless steel surfaces (Figure 8.4).

Copper complexation is discussed in a review article within JACS [18].

8.6.3.1.2 Manufacturing Equipment

The guideline states that the risk associated with manufacturing equipment is generally higher when related to the manufacture of the active due to the typically more aggressive conditions employed in the synthetic process (by

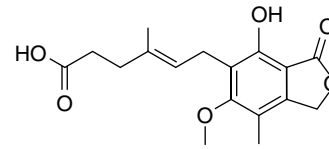


Figure 8.4 Mycophenolic acid.

comparison to typical drug product manufacturing processes). While generally true it is important to consider the key role played by GMP when assessing such risks. Running processes in unsuitable/incompatible equipment simply makes no sense, either in terms of impurity concerns or cost (replacing damaged equipment). GMP will typically involve assessment of equipment compatibility as part of the process accommodation. A significant body of data exists in the literature [19] and with equipment suppliers with which to assess compatibility of specific materials of construction/equipment with common process solvents. Such studies are deliberately designed to show that elemental impurities are not leached from manufacturing equipment into the process liquors and therefore drug substance. This is a clear example of the kind of data-driven, iterative risk assessment process described previously. Furthermore the nature of the risk in terms of the elements involved is clearly understood, as the composition of the equipment is well defined both in terms of elements and their levels within materials such as stainless steel and Hastelloy C22. This knowledge, combined with compatibility assessment, allows for highly effective control over EIs arising from equipment in a controlled process operated under GMP.

This is further augmented by a wide body of data relating to testing of a range of historical APIs that confirms that this is not a significant risk source in controlled processes operated under GMP.

8.6.3.1.3 Processing Aids/Inorganic Reagents

Processing aids such as charcoal, celite, and inorganic reagents such as sodium chloride (NaCl), and cesium carbonate (Cs_2CO_3), are often used in drug substance manufacturing processes and may be used in significant, for example, molar equivalent quantities. The primary concern is that such materials may contain traces of other environmental EIs that may be of concern. Generally the risk is low unless the reagent is used in elevated levels and/or aggressive reaction conditions, for example, extreme pH/high temperatures, are employed.

8.6.3.1.4 Solvents

Solvents in general are very unlikely to contribute elemental impurities to the finished drug substance. Only a subset of solvents involve the direct use of metal catalysts in their manufacture, and moreover the majority of solvents are purified by distillation, which would remove any metal catalyst residues. Further evaluation would generally only be required if a catalyst is used directly in their manufacture and solvent residues are concentrated in the manufacturing process.

Other sources of EIs within the drug substance such as water and container closure systems (CCS) are discussed in the following text.

In relation to control of EIs within the drug substance, what is clear is that this must come through design (quality by design) and cannot simply be tested into compliance (quality by testing). Simple scientific principles can be applied to ensure that elemental impurity levels in the final drug substance are controlled to appropriate levels.

These include appropriate equipment selection/qualification, adoption of suitable GMP processes/procedures; these, combined with the definition and application of appropriate control options for metal catalyst residues, will typically result in the manufacture of drug substances with elemental impurity levels well below ICH Q3D limits.

8.6.3.2 Excipients

In the development of the guideline, it is clear that excipients, particularly mined excipients, were thought to pose a potential substantive risk. It was anticipated that such excipients may contain high and variable levels of environmental metals, particularly class 1 and class 2a metals. The relative risk, based on excipient class, is illustrated in Figure 8.5.

At this stage knowledge of the extent of the risk remains relatively low. However recent studies conducted by the FDA [19] have proved illuminating. The studies involved over 200 samples, spanning a range of excipient types, including plant-derived, synthetic, and mined excipients. Overall results showed little evidence of substantial levels of even the "big 4" (ubiquitous?) elements in mined excipients. Detectable levels of Pb were seen in titanium dioxide but levels <10 ppm are very unlikely to pose a threat in typical formulations and dosing regimens (titanium dioxide is typically used as a minor

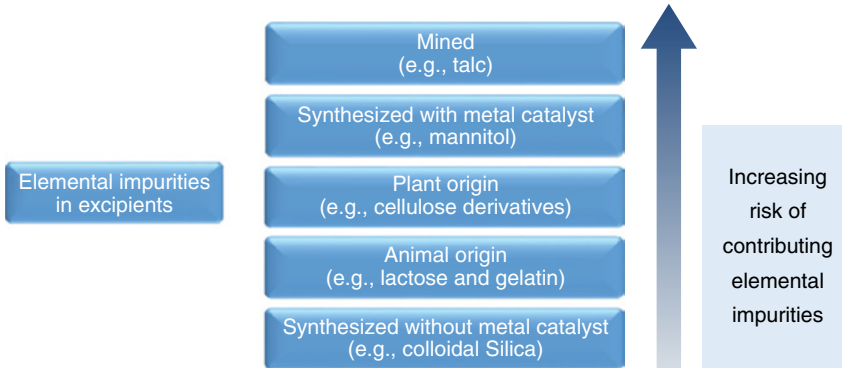

Elemental impurities in excipients
Mined (e.g., talc)
Synthesized with metal catalyst (e.g., mannitol)
Plant origin (e.g., cellulose derivatives)
Animal origin (e.g., lactose and gelatin)
Synthesized without metal catalyst (e.g., colloidal Silica)
Increasing risk of contributing elemental impurities

Figure 8.5 Potential risk associated with excipients based on material source.

component in aqueous film coating materials that are typically applied at 3% w/w of the tablet core). Furthermore across the samples assessed, variability (batch to batch or supplier to supplier) was not significant. In terms of other class 1 elements, Cd levels in magnesium hydroxide and calcium carbonate were seen to exceed option 1 limits—but again it was concluded that it was highly unlikely that these levels would adversely impact on the overall quality of a typical drug product. (These excipients are normally used in quantities significantly lower than 10 g per daily dose.) Metal impurities were found where they might be expected. Class 2A metals were detected at appreciable levels in some mined excipients, for example, ferric oxide samples were seen to contain V, Ni, and Co ~100 ppm, and similarly ferric carbonate contained elevated Ni levels.

Once again it was considered unlikely for such excipients to represent a major percentage of a drug product composition, and hence the class 2 elements are unlikely to exceed permitted daily exposure limits.

Overall very little evidence was seen of the presence of class 2b metals—unless they had been deliberately used in manufacture of the excipient. This validates the advice given in ICH Q3D that evaluation of class 2B metals is only required when such metals have been deliberately used. An example provided within the paper is silicone. Selected silicones were found to contain Pt up to 8 ppm, not altogether unexpected given the use of Pt as a catalyst in the manufacture of the silicone.

Finally several mined excipients were found to contain class 3 metals such as Cr, Mo, Sn, and Ba, but none exceeded option 1 limits.

While the size of the study and the data set it has provided are insufficient at this stage to draw sweeping conclusions, the study does support an overall view that for the majority of drug products, even those employing mined excipients, the risk associated with EIs is likely to be low. This is exemplified in the following anonymized formulation for an existing marketed product. Two mineral colorants, blue lakes, are utilized. Even combined, the percentage composition is well below 1%, and hence the levels of metals present in such materials would have to be in excess of 1000 ppm to have an impact on the overall formulated drug product (Table 8.3).

8.6.3.3 Water

Water is used widely in the manufacture of both the drug substance and the drug product. Indeed in parenteral formulations, especially Large volume parenterals (LVPs), it is the major component of the final drug product. It is therefore clearly a potential source of risk. As with processing equipment, GMP is an integral part of the overall management of this specific risk. The risk is directly related to the quality of water used. During the manufacture of the drug substance, GMP requires the use of a minimum of WHO-standard potable water [20] for stages up to the isolated drug substance and for the final

Table 8.3 Formulation composition and excipient source.

Component	Functionality	Amount per 400 mg tablet (mg)	Percentage in coated tablet	Type (excipient)
Core				
API	Drug substance	400.00	62.64	
Hypromellose	Binder	21.70	3.40	Plant
Microcrystalline cellulose	Diluent	37.20	5.83	Plant
Lactose monohydrate	Diluent	111.50	17.46	Animal
Crospovidone	Disintegrant	43.40	6.79	Synthetic
Magnesium stearate	Lubricant	6.20	0.97	Synthetic
Coating				
Hypromellose	Film former	11.16	1.75	Plant
Titanium dioxide	Pigment	5.55	0.87	Mineral
Triacetin	Plasticizer	1.49	0.23	Synthetic
Blue aluminum lake A	Colorant	0.37	0.06	Mineral
Blue aluminum lake B	Colorant	0.03	0.005	Mineral

isolation, compendial quality, for example, USP purified water [21]. Similarly manufacture of the drug product requires similar exacting standards. The overall issue of the impact of water quality on the EIs was discussed in a USP stimuli article [22]. The following position was articulated:

- The source water used in drug product manufacturing must meet the WHO standard for drinking water [20]. When this source water is further purified in a contemporary plant to generate purified water and/or water for injection (WFI), the elemental impurity levels should be below acceptable concentrations allowed for drug products using option 1 control strategy defined in ICH Q3D.
- As part of standard GMP, water quality should be routinely monitored and the purification system and storage of the water should not reintroduce elemental impurities.

Based on this position, the risk of elevated elemental impurity levels within aqueous-based formulations, even LVPs, is considered negligible. The risk associated with the use of water in the manufacture of the drug substance can also be effectively eliminated through the appropriate use of WHO-standard potable water combined with the use of purified water or water for injection for the final stage in the manufacture of the drug substance, including its isolation.

8.6.3.4 Container Closure Systems (CCS)

One of the potential sources of elemental impurities defined within ICH Q3D is product packaging, often referred to as CCS. The guideline lists a series of factors that should be considered when assessing the risk from such systems, these include

- Physicochemical parameters associated with the drug product primarily focused on liquid formulations; these include hydrophilicity/hydrophobicity, ionic content, pH, temperature (cold chain vs room temperature and processing conditions), contact surface area, container/component composition, terminal sterilization, packaging process, component sterilization, and duration of storage.

In terms of the type of formulation, it is inconceivable that even trace-level contamination would occur where physical contact is limited to solid–solid contact, for example, tablet to bottle or blister packaging. This is consistent with the FDA Guidance for Industry, "Container Closure Systems for Packaging Human Drugs and Biologics" [23], which in relation to extractables and leachables, considers solid-to-solid contact of low risk. This is clearly recognized in the guideline itself with the guideline stating that "It is recognized that the probability of elemental leaching into solid dosage forms is minimal and does not require further consideration in the risk assessment."

The guideline however also states that "for liquid and semi-solid dosage forms there is a higher probability that elemental impurities could leach from the container closure system during the shelf-life of the product." Such a risk does of course depend on the presence of EIs within the packaging material in question. A major review of materials in manufacturing and packaging systems as sources of elemental impurities in packaged drug products was published in 2013 (Jenke et al. [24]). The publication summarized the available literature data for a number of common packaging materials, examining in each case the levels of elemental impurities within the component material (determined by digestion), as well as elemental impurities extracted from the component materials. The data, while fragmentary, are nevertheless compelling and several key conclusions were drawn. While certain materials were found to contain elemental impurities, the presence of the elemental impurity was predominantly associated with deliberate use of metal catalysts, for example, the use of antimony in the manufacture of polyethylene terephthalate (PET). In the case of PET, levels of antimony of around ~50 ppm are typical (full digestion). Some materials were seen to contain measureable levels of certain class 1, and class 2 elements within the component material, for example, cadmium and lead levels up to 100 ppm, were reported in polyvinyl chloride (PVC). However, it is critical in such instances to assess the effective "availability" of the elemental impurity. What is clear from the extraction data presented within the Jenke paper [24] is that extracted elemental impurity levels (under relevant

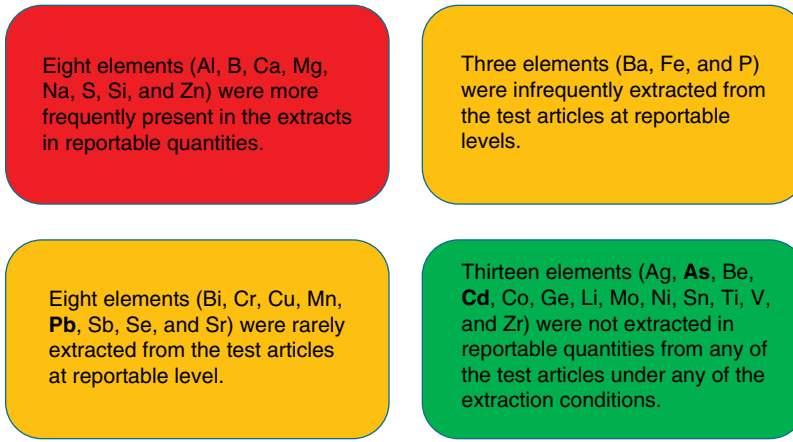

Eight elements (Al, B, Ca, Mg, Na, S, Si, and Zn) were more frequently present in the extracts in reportable quantities.
Three elements (Ba, Fe, and P) were infrequently extracted from the test articles at reportable levels.
Eight elements (Bi, Cr, Cu, Mn, **Pb**, Sb, Se, and Sr) were rarely extracted from the test articles at reportable level.
Thirteen elements (Ag, **As**, Be, **Cd**, Co, Ge, Li, Mo, Ni, Sn, Ti, V, and Zr) were not extracted in reportable quantities from any of the test articles under any of the extraction conditions.

Figure 8.6 Summary of results from Jenke et al. [24] review.

conditions) are typically only a small fraction of the total elemental impurity levels present in the component materials, typically <0.1% of that observed following digestion. Therefore, even when trace levels of certain elements are found in the component material, the available elemental impurity concentration usually represents an extremely low safety risk.

Overall evaluations should thus focus on liquid and semisolid formulations. The first step in any such assessment is to identify any significant potential risk, such as use of a metal catalyst in the manufacture of the materials. Combined with this a literature search to ascertain what, if any, extractables and leachables data may exist for the material in question. Detailed leachable studies should only be required where there is a lack of elemental impurity extractives data for the packaging components concerned or where extractives studies show EI levels above the threshold for toxicological concern. A summary is presented in Figure 8.6. Note the focus of the paper is extractables and leachables and therefore addresses elements outside the scope of ICH Q3D.

8.6.3.5 Drug Product Manufacturing Equipment

In general the risk associated with the manufacture of the drug product is lower than that associated with the drug substance as the conditions used are, as a rule, less chemically aggressive, avoiding the extremes often seen in drug substance manufacture, that is, extremes of pH and/or high temperature and highly reactive reagents. However manufacture of the drug product may still involve the use of solvents or liquid formulations, and therefore consideration needs to be given to the potential risk of EIs arising from contact with manufacturing equipment. In addition, the prevailing conditions can also be physically aggressive, leading to abrasion of manufacturing equipment, for example,

mills or micronizers. Furthermore unlike the manufacture of the drug substance, there is usually no effective mechanism to remove any potential EIs, and the impact is potentially on the entire formulated product rather than a single component.

8.6.3.5.1 Solid Dosage Forms

Even for solid drug product formulations, manufacture may encompass a range of different processes, such as particle size reduction, solid mixing (blending), granulation, tableting (compression), and coating. These may vary significantly in terms of the energy involved in the process.

Typical process operations are described in Table 8.4 along with an evaluation of the level of control required based on the nature of the process and the energy involved. As with the manufacture of the API, GMP plays a key role in assessing the suitability of equipment for the manufacture of the drug product, prior to final selection of processing equipment. Any risk assessment should focus on the specific elements of concern, this being based on the materials of construction. The risk is primarily focused on class 2A metals such as vanadium and nickel, since these elements are commonly found in manufacturing equipment, for example, 316L stainless steel. This although composed predominantly of iron (low toxicity) also contains ~10% w/w nickel). Understanding the drug product manufacturing equipment in terms of materials of construction will be a key factor in completing the risk assessment.

In the case of a high energy process such as micronization, physically abrasive solids may erode parts of the manufacturing equipment over time, in particular the nozzles through which the material is introduced.

The following example illustrates how even in a case where visible abrasion is seen the safety risk was found to be negligible. Figure 8.7 illustrates the wear on nozzles used in the micronization of a particularly abrasive material over time.

The first step in the risk assessment in this example was to determine the extent of the abrasion. This is relatively straightforward and was done by simply weighing the nozzles. Based on this it was concluded that the loss was ~1 g of stainless steel. The nozzles in question had been used to manufacture multiple batches, batch size = 500 kg. However for the purposes of evaluating risk, a worst-case scenario was used, and it was assumed that all loss occurred within 1 batch (unlikely). Overall therefore the maximum level of metal in the drug product = 1 g/500 kg = 2 μg/g.

Molybdenum (Mo) was used as a worst-case guiding substance, based on the composition of the stainless steel and the relative toxicity of the metals present. For the purposes of assessing risk, the ICH Q3D limit for Mo is 3000 μg/day (oral mode of administration). Based on the composition of the stainless steel in question (Mo 3%), this would give a Mo level of 60 ng/g. Where an option 1 limit applied, that is, 300 ug/day (based on 10 g product dose), the level is ≪ permitted levels.

Table 8.4 Typical processing operations used in drug product manufacture and their potential impact and associated cGMP controls.

Unit operation	Kinetic energy	Aggressive conditions	Recommended routine cGMP controls	Remarks
Mixing/ granulation	Low	Dry = no	NA	For example, low equipment rotation/translation speed
	High (shear)	Wet = potentially	Periodic visual inspection of the equipment for abrasion and/or corrosion	Although the likelihood of a potential elemental impurity contribution is increased when moving from low shear mixing to high shear mixing, the overall risk of a significant elemental impurity contribution remains low
Tableting	High	No	Periodic visual inspection, as previously	Normal wear on dyes/punches is unlikely to release any appreciable amount of elemental impurities into the product. However, use of inorganic diluents, for example, abrasive calcium phosphate, can lead to increased punch wear. These formulations typically require higher levels of lubricant to compensate. In addition many tableting processes have a final inspection that includes a de-duster/metal check
Encapsulation	High	No	Periodic visual inspection as previously	—
(Liquid) filling, lyophilization	Low	Potentially	For aggressive, for example, high pH conditions, regular visual inspection of the equipment for corrosion	Effect of actual corrosion, as with tablet punch erosion, unlikely to result in substantive release of elemental impurities at levels of concern into the product
Coating	Low	No	Covered by routine cGMPs, for example, for maintenance, cleaning	—
Particle size reduction	High	Potentially	Despite the high energy, it is not expected that the particle size reduction process will lead to the need for routine drug product testing requirements for elemental impurities associated with the materials of construction of the mill. In the vast majority of cases, routine cGMP will be sufficient	Although the possibility of metal transfer during this process is potentially high due to abrasion, the risk of levels approaching the limits defined in ICH Q3D is extremely low. Such a risk may be evaluated through a mathematical assessment, evaluating the theoretical maximum level of metal possibly transferred during the process, comparing this to permitted limits. Practical evaluation through comparison of the elemental impurity profile of the ingoing material to that of the outgoing material may also be performed. Such an assessment may also take into consideration historical knowledge of similar processes and substances. Such approaches may be used to determine if further controls other than cGMP are required

Source: Kindly reproduced with the permission of Pharmaceutical Technology.



Figure 8.7 Visible comparison of nozzles before and after use.

8.6.3.6 Special Considerations for Biotechnology-Derived Products

The guideline contains a specific subsection relating to biotechnology products. This makes clear that due to the nature of such products and the manufacturing processes and procedures used to produce them, the risk of EI contamination is generally low. This reflects the general absence of the use of metal catalysts in the manufacture and the extensive purification procedures employed including chromatography, ultrafiltration, etc. In the case of antibody drug conjugates (ADCs) where the product includes synthetic structures, a risk assessment is stated as being required. In reality within ADCs the proportion represented by the synthetic entity is often very low; this is generally a highly toxic/highly potent compound and is thus present at very low levels.

The guideline indicates that for biotechnology-derived products, the largest risk factors are manufacturing equipment and environmental factors; however as highlighted previously such risks should be readily manageable through appropriate GMP controls. Therefore the overall risk level for GMP biotechnology products is low.

8.6.3.6.1 Quality Aspects: Biologics

ICH Q3D is of course focused on EIs from the perspective of toxicological safety and assurance that levels of EIs are controlled below permitted limits; however there are also other potential quality-related and even safety-related risks associated with the presence of EIs in the formulated product. For example, metal-catalyzed oxidative degradation of APIs or excipients may occur as a result of the leaching of low levels of metals, particularly in the case of solution-based drug products. Reported examples include iron, nickel, and tungsten oxide [25–28]. In the case of tungsten, this relates to the leaching of

tungsten from a syringe barrel, thus causing protein aggregation. It may be possible to reduce or eliminate such effects through the use of a "scavenging reagent" such as ethylenediaminetetraacetic acid (EDTA) to "mop up" metals.

8.6.4 Evaluate

Based on the initial risk assessment, it may be concluded that there are drug product components that are of significant potential risk, and therefore further evaluation, including specific analytical screening, may be required to better understand the risk. In many instances this is often an iterative process carried out in parallel to the risk identification stage. Where screening is required, it should generally be focused on the identified elemental impurity risks, taking into consideration routes of administration. Generally, multi-element analysis will be employed, typically ICP-MS, although other techniques, for example, XRF, may also be used. Testing at this stage will take the form of a simple screen to inform the overall risk assessment, as opposed to routine QC testing that may be used as part of the overall control strategy. The level of analytical method validation performed therefore must be fit for purpose. For example, screening method validation may consist of some limited spike–recovery testing as opposed to the full ICH Q2 [29] or USP <233> [30] level verification recommended for routine QC test methods.

The next stage in the risk assessment process involves the evaluation of the risks, taking into account any specific data obtained through either analytical screening, supplier information, or other sources, for example, literature data. The IWG training module, specifically module 5, describes the risk assessment output in terms of a series of options, four in total.

Irrespective of the outcome, the risk assessment must be formally documented and comprehensive enough to provide a complete description of the risk assessment process, outlining the contributory factors considered, associated data, and ultimate conclusions of the impact on the final drug product. It should identify any significant risks and the subsequent controls required to manage this, linking directly to the overall control strategy. The guideline provides a benchmark level of 30% of the PDE for an individual element as the basis for defining significance and the need for routine control.

Another important factor to take into consideration is variability, including variability associated with

- The analytical method.
- Levels of EIs within a specific source.
- Levels of EIs within the formulated product.
- It is has long been postulated that the most likely source of variability in terms of levels will be mined excipients. At present the extent and level of risk associated with such materials is still to be properly determined; however as already described, results from preliminary studies [31] are encouraging both in terms of the low levels seen and limited variability observed.

Clearly the impact is directly proportional to the level of a particular component within the formulated product. Certainly if the levels of mined excipients used in the drug product are low and/or the overall drug product daily dose is low, then the level of variation suspected across mined excipients will not have a significant impact.

It should be noted that the guideline does not however definitively describe a specific format for a risk assessment summary, hardly surprising given the case-by-case nature of such assessments; thus the onus is on the applicant to define what is appropriate.

8.6.5 Control of Elemental Impurities

Although Figure 8.8 illustrates a series of outcomes of the evaluate phase, in reality and experience to date, there are two outcomes, either no significant risk is identified or a risk is identified and specific control is required. Where control is required, for example, drug substance, excipient, or drug product, this is likely to require QC testing, aligned to an appropriate specification. Depending on the source of the EI risk, this testing may be performed on either a component of concern or the final formulated product. For example, in the case where levels of a specific metal catalyst associated with the drug substance are identified as a risk, testing would typically be focused on the drug substance to ensure timely control.

When the risk assessment concludes that there is no substantive risk and all elemental impurities are controlled below 30% PDE, there is still a need to maintain effective control through the overall quality system, aligned to the principles outlined in ICH Q10 [32] including appropriate use of standard cGMPs and change control. This needs to also take into consideration other factors such as:

- Security of external supply chain along with a quality history (e.g., audit history/levels of complaints/recalls, etc.) for each vendor
- Control of current vendor elemental impurity specifications and elemental impurity reporting on ingredient certificates of analysis
- Security of internal supply chain

Of particular importance during the life cycle of the product is change control. A change proposal should be created for any commercial supply changes with the potential to impact product quality. Each change proposal should be reviewed for the potential to introduce new, or increased levels of EIs. Appropriate documentation of the original risk assessment position will facilitate review of future changes. Processes such as the FMECA risk assessment process illustrated in the example in the following text, can be updated to reflect any significant changes to the product risk profile.

In order to effectively manage the interface between component suppliers and drug product manufacturers, IPEC has developed a template questionnaire (accessible via IPEC Americas site).

Figure 8.9 shows the front page of the questionnaire of a version used by AZ based on the IPEC template.

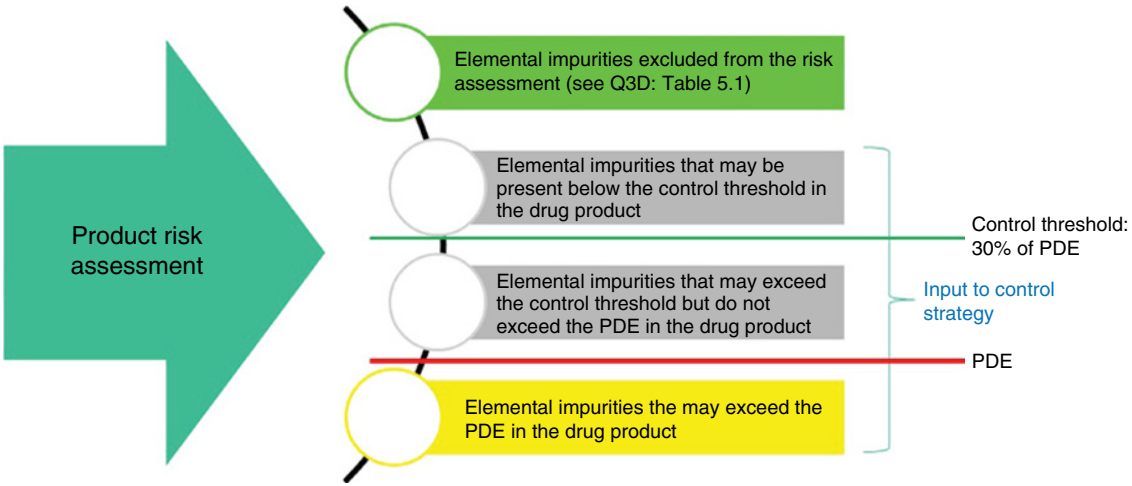

Product risk assessment
Elemental impurities excluded from the risk assessment (see Q3D: Table 5.1)
Elemental impurities that may be present below the control threshold in the drug product
Elemental impurities that may exceed the control threshold but do not exceed the PDE in the drug product
Elemental impurities the may exceed the PDE in the drug product
Control threshold: 30% of PDE
PDE
Input to control strategy

Figure 8.8 Relationship between product risk assessment outcomes and control strategy.

Questionnaire for Identification of Potential Elemental Impurities

Supplier Name:	**Supplier Phone Number:**
Supplier Address:	**Supplier Email Address:**
Manufacturer (if different than Supplier):	**Date Form Filled Out:**

AstraZeneca requires information on the elemental impurity profile of the listed material, in order that we can confirm that our manufactured pharmaceutical product complies with the requirements of the International Conference on Harmonization Guideline for Elemental Impurities ICH Q3D(1), the EMEA guideline on "the specification limits for residues of metal catalysts or metal reagents" (EMEA/CHMP/SWP/446/2000)(2) and the US Pharmacopeia General Chapters <232>/<233>(3).

Please complete this document giving due consideration to all of the added starting materials and manufacturing equipment used during the manufacturing process of the named material.

Identify elemental impurities in (*to fill-in Material Name*) that are likely to be present. If likely to be present, identify expected concentration (or range), analytical method used, and limit of detection, if known

Please complete a separate form for each material

Material Name:_____ Material Grade:_____

Material Number:_____

Material source: ☐ Mineral ☐ Mineral derived ☐ Plant ☐ Plant derived ☐ Synthetic ☐ Fermentation derived

Other (explain):_____

Is there any history of this material failing to meet heavy metals test USP <231>, EP 2.4.8 or JP 1.07 limits?

Yes ☐	No ☐	Material not tested ☐

Figure 8.9 Modified IPEC questionnaire.

Elemental Impurity		ICH Q3D Class	Metal intentionally used in manufacturing process?*		Likely to be Present*			If Known, Please Identify the Expected Concentration /Units (or Range)	Is the elemental impurity controlled via a specification test? Y/N (limit)	Analytical Method Used (and Limit of Detection if Available)	*If yes, please comment on source of elemental impurity, process understanding & frequency of testing
Arsenic (inorganic)	As	1	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Cadmium	Cd	1	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Mercury (inorganic)	Hg	1	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Lead	Pb	1	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Cobalt	Co	2A	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Vanadium	V	2A	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Nickel	Ni	2A	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Thallium	Tl	2B	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Gold	Au	2B	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Palladium	Pd	2B	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Iridium	Ir	2B	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Osmium	Os	2B	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Rhodium	Rh	2B	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Ruthenium	Ru	2B	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Selenium	Se	2B	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Silver	Ag	2B	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Platinum	Pt	2B	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Lithium	Li	3	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				

Figure 8.9 (Continued)

Antimony	Sb	3	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Barium	Ba	3	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Molybdenum	Mo	3	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Copper	Cu	3	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Tin	Sn	3	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Chromium	Cr	3	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Iron	Fe	N/A	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Zinc	Zn	N/A	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Manganese	Mn	N/A	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Aluminium	Al	N/A	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Boron	B	N/A	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Tungsten	W	N/A	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Potassium	K	N/A	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Calcium	Ca	N/A	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Sodium	Na	N/A	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Magnesium	Mg	N/A	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Other metals << Name>>		N/A	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				
Other metals << Name>>		N/A	Yes ☐	No ☐	Yes ☐	No ☐	Unknown ☐				

Figure 8.9 (Continued)

The sections described previously reflect the basic principles outlined in the guideline in terms of the process of risk assessment; the next section within the guideline itself (section 7) outlines options by which the specific drug product risk assessment may be conducted and the control strategy defined.

8.6.6 Converting between PDES and Concentration Limits

The PDEs illustrated in Table 8.1 are reported in dose terms, that is, micrograms per day (μg/day). It is however often convenient to convert the PDE into concentrations as a means to evaluate elemental impurity levels (μg/g) in drug products or their components. The guideline describes a series of options for converting between PDE and concentration limits. Some are useful during the "analyze" phase (in order to determine appropriate sample preparation concentrations in order to achieve required limits of detection/quantification), and some during the evaluate or control phase (establishing concentrations of elemental impurities in drug products or components that assure that the drug product does not exceed the PDEs). It makes clear that an applicant may utilize any of these options as long as the resulting permitted concentrations do not exceed the PDEs.

8.6.6.1 Option 1: Common Permitted Concentration Limits of Elements across Drug Product Components for Drug Products with Daily Intakes of Not More Than 10 Grams

This option assumes the daily intake (amount) of the drug product is 10 g or less. Using Equation 8.1 in the following text, and a daily intake of 10 g of drug product, this option calculates a common permissible target elemental concentration for each component in the drug:

$$\text{Concentration}\ (\mu g/g) = \frac{\text{PDE}\ (\mu g/\text{day})}{\text{daily amount of drug product}\ (g/\text{day})} \qquad (8.1)$$

The benefit of such an approach is that if all the components in a drug product do not exceed the option 1 concentrations for all target elements identified in the risk assessment, then all these components may be used in any proportion in the drug product. This makes the option 1 limit a useful target for assessment of generic excipients. What is important to appreciate is that an excipient that exceeds the option 1 limit can be used in the majority of formulations, provided the levels of elemental impurities are compensated (lower) in the other formulation components *or* the daily dose of the drug product is <10 g. In reality some materials, for example, mined excipients, are unlikely to achieve the option 1 limits. Rigidly applying this approach could lead to unachievable limits that in reality are not required. Flexibility in approach is necessary to avoid ruling out common useful excipients.

8.6.6.2 Option 2a: Common Permitted Concentration Limits across Drug Product Components for a Drug Product with a Specified Daily Intake

This option is effectively the same as option 1, except that the actual drug product daily intake is used as opposed to an assumed generic dose of 10 g. This option provides more flexible limits for the majority of drug products that are dosed at <10 g/day.

Options 1 and 2a are useful for setting common target limits for drug products or components during the identify/analyze stages of a risk assessment when actual levels of elemental impurities or quantitative formulation composition may be unknown.

8.6.6.3 Option 2b: Permitted Concentration Limits of Elements in Individual Components of a Product with a Specified Daily Intake

This option allows the applicant to define permitted concentrations based on the distribution of elements within individual components; the advantage of such an approach is that it allows higher concentrations for specific elements to be established within certain components, this being effectively balanced by absence or lower levels in other components. The maximum expected level in dose terms (μg) of the elemental impurity in the final drug product is then calculated, taking into consideration the permitted concentration of the element in the component in question along with the amount of the component in mass terms present in the formulated product. The calculation is presented in the following text:

$$\text{PDE}\ (\mu\text{g/day}) \geq \sum_{k=1}^{N} C_k \cdot M_k$$

where

k = An index for each of N components in the drug product
C_k = Permitted concentration of the elemental impurity in component k (μg/g)
M_k = Mass of component k in the maximum daily intake of the drug product (g)

The total dose of the elemental impurity in the drug product should comply with the PDEs given in the guideline (see Table 8.1), unless justified according to other relevant sections of this guideline, for example, short-term dosing/indication. Critically the guideline makes clear that if the risk assessment has determined that a specific element is not a potential impurity in a specific component, there is no need to establish a quantitative result for that element in that component (it can be assumed to be zero for the purposes of the calculation).

This option is particularly useful for evaluating analytical data, following the identify/analyze stages of the risk assessment, or for setting tailored

Component	Category	Quantity (mg/tablet)	Dose "x" tablets (mg/day)	Arsenic in component ug/g		As ug in daily dose of formulation		Lead in component ug/g		Pb ug in daily dose of formulation		Mercury in component ug/g		Hg ug in daily dose of formulation		Cadmium in component ug/g		Cd ug in daily dose of formulation	
		x =	2	Total	Bio Acc	Total	Bio Acc	Total	Bio Acc	Total	Bio Acc	Total	Bio Acc	Total	Bio Acc	Total	Bio Acc	Total	Bio Acc
Tablet core:																			
Active	Synthetic	100	200	0.00		0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.00
mannitol	Synthetic	120	240	0.00		0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.00
calcium phosphate	Mineral	65	130	0.11		0.01	0.00	0.02		0.00	0.00	0.00		0.00	0.00	0.03		0.00	0.00
modified starch	Plant derived	10	20	0.00		0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.00
cellulose	Plant derived	10	20	0.00		0.00	0.00	0.09		0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.00
magnesium stearate	Synthetic	3	6	0.02		0.00	0.00	0.01		0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.00
titanium dioxide	Mineral	2	4	0.03		0.00	0.00	2.41		0.01	0.00	0.01		0.00	0.00	0.04		0.00	0.00
talc	Mineral	1	2	0.17		0.00	0.00	0.19		0.00	0.00	0.01		0.00	0.00	0.00		0.00	0.00
polyethylene glycol	Synthetic	1	2	0.00		0.00	0.00	1.00		0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.00
ferric oxide red	Mineral	0.1	0.2	0.66		0.00	0.00	0.37		0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.00
purified water*		NA																	
Total Tablet weight		312.1	624.2																
Total element				As		0.02	0.00	Pb		0.02	0.00	Hg		0.00	0.00	Cd		0.00	0.00

* NOTE: PF 39.1 Revision Process stimuli "Elemental Impurities in Pharmaceutical Waters" excludes analysis of compendial water meeting monograph conductivity requirements

Permissible Limits	As	Pb	Hg	Cd
Formulation	Q3D	Q3D	Q3D	Q3D
Oral PDE	15	5	30	5
Parenteral PDE	15	5	3	2
inhaled PDE	2	5	1	2

Figure 8.10 Daily dose calculator.

specifications for components of the drug product (where a need for control is identified following the risk assessment). It can also be useful for setting target limits for drug product components during the identify/analyze phase when it is clear that only one component of the drug product is likely to contain a specific elemental impurity of interest, for example, a metal catalyst.

The coalition for rational implementation of elemental impurity guidelines has in partnership with IPEC Americas developed a calculation tool to facilitate the option 2B calculations (accessible through IPEC Americas website); an example of the template is shown in Figure 8.10. Also included on the website concerned is an instruction manual on how to use the simple tool.

Included within the tool are both blank templates and predefined templates illustrating a wide range of formulation types ranging from simple tablets to large volume parenterals.

8.6.6.4 Option 3: Finished Product Analysis

This option involves measurement of the concentration of each element in the final drug product. Typically Equation 8.1 is used with the maximum total daily dose of the drug product (g/day) to calculate a maximum permitted concentration of the elemental impurity (μg/g). While apparently the simplest option, this does not effectively align with the core risk assessment principles of the guideline and when used in isolation represents little more than quality by testing. A major flaw with option 3 is that were the drug product to fail, it would give no indication as to the root cause.

In reality, most companies are likely to encapsulate aspects of each option into their risk assessment processes. For example, target levels to frame analysis of a generic excipient may be calculated using option 1 limits. Levels measured in individual drug product components may be compared with the PDE using an option 2A or option 2B approach, and during the development phase, option 3 testing of any high risk drug products may be preferred to component analysis and results fed back into the overall risk assessment to refine the risk assessment and define the long-term control strategy.

8.7 Speciation

The guideline clearly states that an applicant is not expected to provide definitive data relating to the exact species, for example, valance state of an elemental impurity, unless the applicant specifically seeks to justify higher or lower limits based on such factors. The likely form is effectively addressed through the individual risk assessments described in Appendix 3 of the guideline. One specific example relates to mercury where in the case of materials derived from a marine environment, the risk assessment needs to consider the risk posed by organomercury impurities.

8.8 Analytical Procedures

The guideline does not provide specific guidance in terms of what procedures/techniques to use nor does it provide any effective guidance regarding associated validation criteria. It instead refers to pharmacopoeial procedures. Such requirements are under development via the pharmacopoeial discussion group (PDG), USP acting as coordinating body. Currently EDQM and USP [29] advocate similar instrumental procedures and verification/validation criteria.

8.9 Training Modules

In April 2016 a series of training modules were published via the ICH Q3D IWG [8]. These modules were designed to augment the guideline and assist practical implementation. It is important to note that they do not seek to provide additional guidance but exemplify the principles outlined in ICH Q3D. These are organized into a series of 10 modules in total:

Module 0: Overview
Module 1: Different Routes of Administration
Module 2: Justification of Levels Greater Than the PDE
Module 3: Non-ICH Elements
Module 4: Large Volume Parenterals
Module 5: Risk Assessment
Module 6: Control
Module 7: Conversion of PDEs into Concentrations
Module 8: Case Studies
Module 9: FAQs

8.9.1 Module 0

This provides an overview of the modules, included within this is a very useful flow diagram (Figure 8.11), highlighting the anticipated overall process from the risk assessment through to definition of control strategy.

Modules 1–4 Cover Toxicology Aspects

8.9.2 Module 1: Different Routes of Administration

Module 1 seeks to provide guidance with respect to how the limits defined in ICH Q3D can be modified and extrapolated to apply to other routes of administration beyond those described in ICH Q3D, that is, oral, parenteral, and inhalation. The module sets out a series of principles based on a start point of the oral PDE. It also describes the need to take into account local effects and the need to assess bioavailability relating to the route concerned. Where the

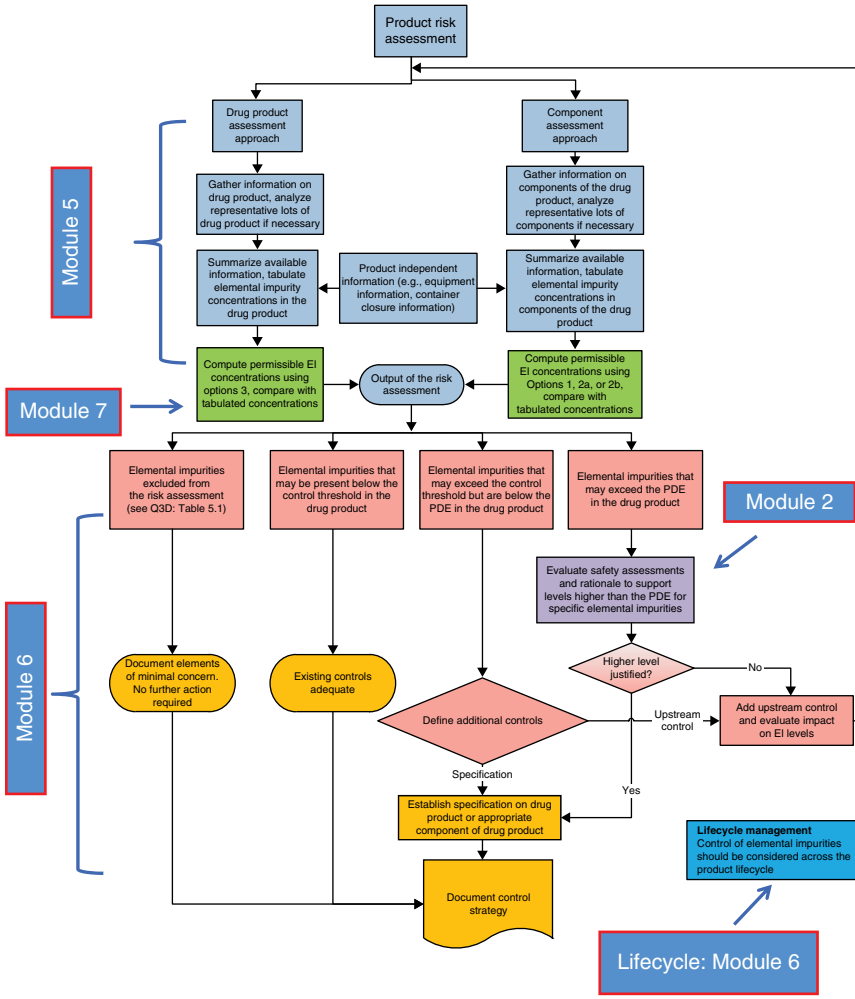

Product risk assessment
Drug product assessment approach
Component assessment approach
Module 5
Gather information on drug product, analyze representative lots of drug product if necessary
Gather information on components of the drug product, analyze representative lots of components if necessary
Summarize available information, tabulate elemental impurity concentrations in the drug product
Product independent information (e.g., equipment information, container closure information)
Summarize available information, tabulate elemental impurity concentrations in components of the drug product
Compute permissible EI concentrations using options 3, compare with tabulated concentrations
Module 7
Output of the risk assessment
Compute permissible EI concentrations using Options 1, 2a, or 2b, compare with tabulated concentrations
Elemental impurities excluded from the risk assessment (see Q3D: Table 5.1)
Elemental impurities that may be present below the control threshold in the drug product
Elemental impurities that may exceed the control threshold but are below the PDE in the drug product
Elemental impurities that may exceed the PDE in the drug product
Module 2
Evaluate safety assessments and rationale to support levels higher than the PDE for specific elemental impurities
Module 6
Document elements of minimal concern. No further action required
Existing controls adequate
Define additional controls
Higher level justified?
No
Upstream control
Add upstream control and evaluate impact on EI levels
Specification
Yes
Establish specification on drug product or appropriate component of drug product
Lifecycle management
Control of elemental impurities should be considered across the product lifecycle
Document control strategy
Lifecycle: Module 6

Figure 8.11 Relationship between training modules and EI risk assessment process. *Source:* ICH Q3D [1]. Courtesy of ICH.

bioavailability differs substantially from that observed orally, a correction factor is applied.

The module observes that oral bioavailability overestimates dermal bioavailability; however it suggests that the use of penetration enhancers (e.g., some transdermal patches) may necessitate a lower starting point than the oral PDE. This is difficult to understand given the evidence presented in Teasdale et al. [33] publication that showed even in the case of a deliberately designed dermal product, based on lidocaine, the levels of active observed systemically were six

times lower than the level observed following oral administration. It is likely that the difference would be significantly greater in case of a metal ion. Indeed Teasdale et al. reviewed the data available for class 1 and class 2A metals, showing dermal absorption of metals to be low, typically well below 10%, for example, levels of arsenic and cadmium absorbed from soil being 3% for arsenic and 1% for cadmium.

The module also presents a series of case studies covering dermal and facial products, ear drops, and eye drops; these are a useful guide.

8.9.3 Module 2: Justification for Limits > PDE

The module directly references section 3.3 of the guideline and highlights again the specific circumstances whereby limits higher than the standard PDE may be justified (see Section 8.4.1 of this chapter). The approach described in the module involves modification of the PDE calculation, specifically *F*2 (as described in Section 8.4, *F*2 = a factor to account for variability between individuals). This is done through splitting the factor into two, one addressing F2.1 TK and the other F2.2 TD, both having a range between 1 and 3.16.

While potentially attractive the issue is availability of data. In the case of F2.1, half-life data are needed. Neither the module nor the case studies provided within, suggest when/how modifications can be made to F2.2.

Factors *F*3 (duration) and *F*4 (severity) can be modified. Interestingly *F*4 does not necessarily relate to duration.

8.9.4 Module 3: Developing an Acceptable Level for an Elemental Impurity Not in Q3D

This is a short module based around the example of sulfur. The approach described is based on an evaluation of the available literature and a PDE calculation.

8.9.5 Module 4: Large Volume Parenterals

The guideline makes the following statement pertaining to parenterals:

> Parenteral drug products with maximum daily volumes up to 2 liters may use the maximum daily volume to calculate permissible concentrations from PDEs. For products whose daily volumes, as specified by labeling and/or established by clinical practice, may exceed 2 liters (e.g., saline, dextrose, total parenteral nutrition, solutions for irrigation), a 2-liter volume may be used to calculate permissible concentrations from PDEs.

Module 4 outlines the basis for this, specifically the upper limit of 2 l. This is based on the methodology developed by Holliday and Segar [34] that is still

used as the basis for determining the average daily fluid replacement need for hospitalized patients. This is based on the volume of fluid needed to replace every calorie expended. The module also includes example case studies including one relating to sodium chloride injection, USP. What is interesting about this specific example is that the risk assessment described is based almost entirely on first principles and available data, in this case published data relating to levels of class 1 metals in sodium chloride.

Modules 5–7 cover Chemistry (quality) aspects.

8.9.6 Module 5: Risk Assessment

Module 5, relating to risk assessments, discusses the key role of GMP in assessing risk—this is an important and a helpful point relating to both API and drug product manufacture. It emphasizes the importance of design and qualification and maintenance procedures.

Specifically in relation to the risk arising from manufacturing equipment, it is generally accepted that the risk associated with API manufacture tends to be higher due to the often more chemically aggressive nature of API manufacturing procedures compared with drug product manufacturing. It even suggests monitoring the drug substance for potential impurities arising from manufacturing equipment (e.g., stainless steel—Cr, Mn, Mo, V, Ni). This is a concern and any such investigation should first be preceded by a risk assessment. Indeed as part of GMP, compatibility should be assessed proactively; this is a key element of the process accommodation procedure, rather than rely on screening to verify retrospectively.

Module 5 highlights that metal catalysts used in the synthesis of drug substances represent a significant potential source of elemental impurities, especially if used in the latter stages of synthesis. It also states that:

> "*Knowledge of potential elemental impurities in synthetic steps prior to the final drug substance may provide information that can assist in the preparation of the risk assessment*".

It is, though open to interpretation as to what this means in practice, how far back in the synthesis an applicant needs to go.

Another interesting point made in the module is the potential for "platform" risk assessments. This is the concept of a risk assessment applicable across a series of products. One such platform may be, for example, oligonucleotides. In such instances, where the manufacturing process in terms of reagent type, equipment, and process conditions are similar irrespective of the precise end product, it should be possible to conduct an assessment based on one process and for this to be relevant/transposable to comparable processes.

8.9.7 Module 6: Control of Elemental Impurities

Module 6 raises the importance of control across the product life cycle. In the context of the manufacture of the API and DP, this requires oversight and governance over changes to the process that may affect the risk assessment, for example, change in catalyst load. Such changes may require a reevaluation and possibly confirmatory testing. Another point emphasized in the module is that routine testing of class 1 metals, that is, arsenic (As), mercury (Hg), cadmium (Cd), and lead (Pb), is NOT required unless there is an identified risk. This is a very important and helpful point clearly reiterating the core principle of ICH Q3D that any control strategy should be based on the risk assessment.

8.9.8 Module 7: Calculation Options

Module 7 relates to section 7 of the guideline and the calculation options described within it. This relates to the conversion of PDEs into concentrations and the subsequent use of the calculated concentrations to evaluate the significance of predicted levels of elemental impurities in drug products/components. Case study examples are provided to illustrate the options available, that is, option 1, option 2a, option 2b, and option 3, these being articulated through examples of oral, parenteral, and inhalation formulations. It is important to understand that the examples are theoretical and the levels described in API, excipients and CCS, are not based on actual data. One area that many find surprising regarding the case studies is the attempt made in all three examples of an option 2b approach to take actual measured values and then relate that back to the individual components to set individual specifications at the component level. Many question the necessity of such an approach, especially as evidence to date does not support a view that it is necessary to exercise specific control over elemental impurities at a component level.

8.10 Case Study

The following example relates to a solid oral dose form, the composition of which is described in Table 8.5

The exact composition of the formulation is shown in Table 8.6.

8.10.1 Step 1: Identify

The first step in the process is to evaluate scope; this can be instigated by first considering factors such as

- Based on composition what is the highest risk source?
  - Can limits be modified based on factors such as disease area and duration of treatment?
- What elements are within scope?

Table 8.5 Case study formulation.

Product	
Dose form	Tablet
Strength	200/400 mg active
Therapeutic target (why patients take this product)	Osteoarthritis
Dosing regimen (frequency and duration of dosing)	Daily, one tablet
Maximum daily dose of active	400 mg of active
Mass of dosage unit	638.6 mg
Route of administration	Oral
USP monograph for product	No
Site of manufacture	GMP
Packing site	GMP

Table 8.6 Formulation composition.

Component	Functionality	Amount per 400 mg tablet (mg)	Percentage in coated tablet	Type (excipient)
Core				
API	Drug substance	400.00	62.64	
Hypromellose	Binder	21.70	3.40	Plant
Microcrystalline cellulose	Diluent	37.20	5.83	Plant
Lactose monohydrate	Diluent	111.50	17.46	Animal
Crospovidone	Disintegrant	43.40	6.79	Synthetic
Magnesium stearate	Lubricant	6.20	0.97	Plant/synthetic
Coating				
Hypromellose	Film former	11.16	1.75	Plant
Titanium dioxide	Pigment	5.55	0.87	Mineral
Triacetin	Plasticizer	1.49	0.23	Synthetic
Blue aluminum lake	Colorant	0.37	0.06	Mineral
Blue aluminum lake	Colorant	0.03	0.005	Mineral

In terms of the product in question, this has a relatively high dose in terms of the active, maximum 400 mg; therefore this represents the highest risk at least in terms of percentage composition. As a treatment for a chronic, nonlife-threatening condition, it is unlikely that any modulation of permissible limits will be acceptable, and finally as an oral treatment, the risk assessment should

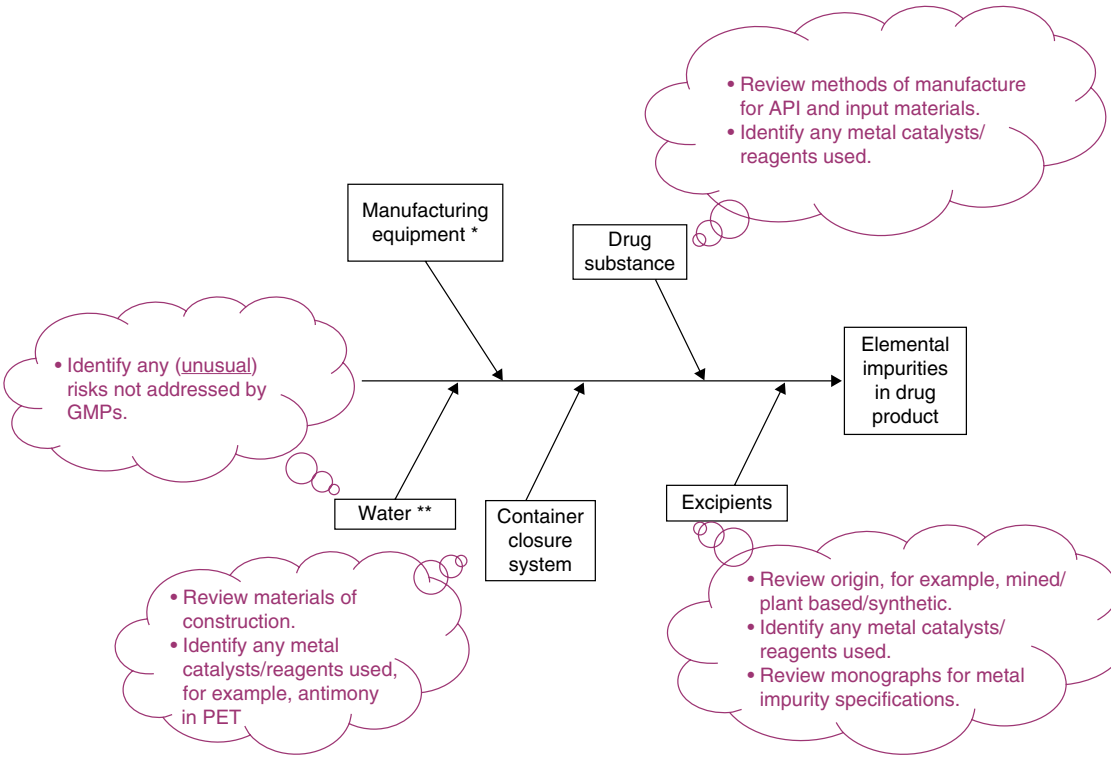

• Review methods of manufacture for API and input materials.
• Identify any metal catalysts/ reagents used.
Manufacturing equipment *
Drug substance
• Identify any (unusual) risks not addressed by GMPs.
Elemental impurities in drug product
Water **
Container closure system
Excipients
• Review materials of construction.
• Identify any metal catalysts/reagents used, for example, antimony in PET
• Review origin, for example, mined/ plant based/synthetic.
• Identify any metal catalysts/ reagents used.
• Review monographs for metal impurity specifications.

Figure 8.12 Preliminary risk assessment.

focus on class 1, class 2A, and any class 2B/other metals used in the manufacturing process.

The next stage—relating to the fishbone diagram—is to start to evaluate potential high risk sources; Figure 8.12 shows the kind of questions that could be considered.

Consider sources in more detail.

8.10.1.1 Manufacturing

In the case of both the drug substance and drug product, they are manufactured within a GMP facility. GMP should define minimum standards that will mitigate certain risk factors. This should typically include

- Equipment compatibility—this particularly in the case of the API ensuring chemical compatibility (including potential chelation with API) between the process and the equipment used
- Cleaning and inspection
- Use of pharmacopoeial grade water

Through such processes the impact of equipment and utilities can be effectively eliminated.

8.10.1.2 API

The risk arising from the API is discussed in detail earlier in section 8.5.2.3; in relation to the case study, the manufacturing process is described in Figure 8.13.

In this specific case a metal catalyst, Pd, is used in the manufacture of the RSM. In terms of process compatibility as part of the accommodation process, specific attention was paid to the step employing *N*-bromosuccinimide (NBS) due to its corrosive nature.

8.10.1.3 Drug Product Manufacturing Process

The manufacturing process is described in Figure 8.14.

The earlier section 8.6.3.5 and table 4 examine the risk posed by the drug product manufacturing process. In relation to the case study, the highest potential risk was defined as Step 2: High Shear Wet Granulation. This was assessed as low risk, based on the use of water, short residence times, and lack of any evidence of corrosion on inspection, and no further evaluation was made. This is entirely consistent with section 5.2 of the guideline that states that *Risk can be reduced through process understanding/equipment selection/qualification and GMP processes.*

8.10.1.4 Packaging

Drug Substance Packaging

Drug substance is stored in double low density polyethylene bags individually closed with plastic tie wraps. The closed bags are stored inside a rigid outer container/drum.

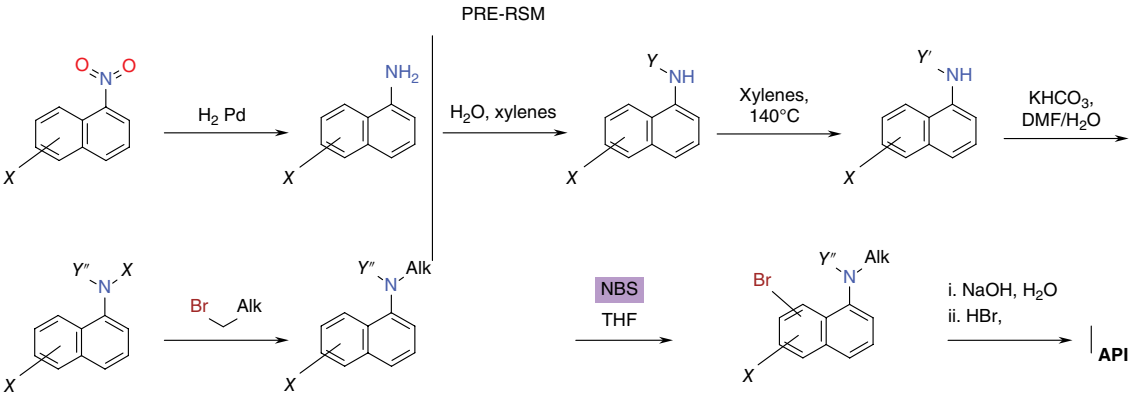


Figure 8.13 API manufacturing process.

Formulation and components		Unit operations	
API Lactose Microcrystalline cellulose Crospovidone Hypromellose Hypromellose Purified water	→ →	**Stage 1: Dry mix** High shear wet granulator **Stage 2: High shear wet granulation** High shear wet granulator	
		↓	
		Stage 3: Wet milling Screening mills **Stage 4: Fluidized bed drying** Direct heating, fluidized solid bed	
		↓	
		Stage 5: Milling Screening mill	
		↓	
Crospovidone Magnesium stearate	→	**Stage 6: Blending** Diffusion mixers (tumble) **Stage 7: Lubrication** Diffusion mixers (tumble)	
		↓	
		Stage 8: compression Tablet press	
		↓	
Film coat	→	**Stage 9: Film coating** Pan coating **Stage 10: Packing**	

Figure 8.14 Manufacturing process.

Drug Product Packaging
Tablets are presented as blister packs formed from unplasticized PVC film laminated to polychlorotrifluoroethylene (PCTFE) and sealed to push-through blister foil.

Both the API and formulated DP are effectively solid dosage forms. ICH Q3D specifically states:

> *Section 5.3—Probability of elemental impurities leaching into solid dosage forms is minimal and does not require further consideration in the risk assessment.*

This is further augmented by published data relating to CCS. Jenke et al. [24] summarized literature data for a number of common packaging materials.

Trace levels present within the component material, for example, cadmium and lead levels up to 100 ppm, were reported in PVC. However this relates to full digestion. In practice effective "availability" of the elemental impurity needs to be considered. Typically extraction levels were <0.1% of that observed following digestion. Therefore, even when trace levels of certain elements are found in the component material, the available elemental impurity concentration represents an extremely low safety risk.

8.10.1.5 Utilities

Utilities are such low risk that they are effectively out of scope where GMP controls are in place. The guideline specifically states that

> Water. "The risk of inclusion of elemental impurities from water can be reduced by complying with compendial (e.g., European Pharmacopoeia, Japanese Pharmacopoeia, US Pharmacopeial Convention) water quality requirements, if purified water or water for injection is used in the manufacturing process(es)."
>
> Air. "Air is not likely to present a substantive risk; furthermore, air quality can also be managed through proper GMPs via use of HEPA filtered air, etc. No specific assessment is therefore generally required."

8.10.1.6 Excipients

Excipients theoretically are considered to pose a potential risk; this is particularly true in the case of mined excipients where there is also considered to be a risk of variability. In the context of the example case study, examination of the composition (see Table 8.6) shows that the mined excipients are present at individual levels <1% of the overall composition. Therefore the overall risk rating for these excipients in this drug product is low. However the biggest challenge at present is the absence of data to verify this risk assessment position.

8.10.1.7 Risk Scoring

Based on the examination of the factors described, a risk assessment was conducted.

The example shown illustrates how a FMECA-type approach may be applied. A fundamental principle of the FMECA approach is stepwise assessment of risks in terms of probability/severity and detectability.

The FMECA is shown in Figure 8.15.

8.10.1.7.1 API

As a deliberately added metal catalyst probability for palladium is scored as 10 (scale of 1–10); however this relates to the manufacture of the starting material therefore severity was scored as 1 due to multiple purification steps between use and API supported by impurity tracking data. The detectability score for the Pd catalyst is 0.1 because the Pd residues were controlled by an intermediate specification upstream of the drug substance.

8.10.1.7.2 Formulated Product

Despite the fact that in terms of the overall composition, the levels of mined excipients were low, <1%, the absence of data led to these being provisionally scored as high risk. Even in the case of low risk excipients, for example, lactose, the absence of substantive data drew us to conclude that to assess the risk appropriately, data would be needed. It is though ultimately hoped that as understanding and available data grow, it may be possible to assess such risks without extensive testing.

8.10.2 Step 2: Evaluate

Based on the preliminary risk assessment some elemental impurity screening was proposed, this is described in Table 8.7.

In the specific case of crospovidone, data was provided by the vendor including confirmation that no catalyst was used.

ICP-MS data was collected for those high/medium risk sources of metal impurities. Three commercially representative batches of each material are analyzed in order to assess variability. *No data are shown for the simple reason that no class 1 or class 2a elements were observed in any of the excipients tested.*

8.10.3 Control

Based on the risk assessment and the analytical data generated, it was concluded that no specific controls were required.

This example is typical of the outcomes to date and mirrors expectations that in all but a small number of cases products manufactured in appropriate GMP facilities, using high quality input materials are unlikely to pose a substantive risk.

Unit Operation/Source of Metal	Failure Mode, (Material)	Failure Effect (Metal of interest)	PDE/ product daily dose (ug/g)	Typical range, (ug/g)	Probability (Risk) Factor	Severity	Criticality Number	General Comments/Control Strategy	Current control level (ug/g)	Detectability	Risk Priority Number	Action
Drug substance												
		environmental metal impurities			5	5	25	Inorganic reagents used in later stages of synthesis.		1	25	Test for environmental metals
		Pd			10	1	10	Residues controlled to 10ppm in drug substance intermediate X (typical levels 0.3ppm).	10 ppm	0.1	1	
		Hg			5	5	25	Potentially introduced with sodium hydroxide		1	25	
Excipients	Hypromellose											
		environmental metal impurities		2.3 <20ppm heavy	5	5	25	USP, Ph.Eur and JP 20 ppm heavy metals	20 ppm	1	25	Test for environmental metals.
		Sn		100213 <0.6 ppm	5	1	5	Identified as likely to be present in supplier survey (<0.6 ppm in 12 samples by ICP-OES).		1	5	
	Microcrystalline Cellulose											
		environmental metal impurities		2.3 <40ppm	5	5	25	USP, Ph.Eur and JP 10 ppm heavy metals	10 ppm	1	25	Test for environmental metals.
	Lactose monohydrate											
		environmental metal impurities		2.3 <1ppm	5	5	25	USP, Ph.Eur and JP 5 ppm heavy metals	5 ppm	1	25	Test for environmental metals.
	Crospovidone											
		environmental metal impurities		23.5 <10ppm	1	5	5	USP and Ph.Eur 10 ppm heavy metals	10 ppm	1	5	
		Catalyst (?)			10	5	50	Pharmaceutical excipient feedback suggests that a catalyst is used. Contact supplier.		1	50	Contact supplier to confirm if a metal catalyst is used.
		Lead		7.8 <5ppm	1	1	1	Test in on current supplier CofA		1	1	
		Arsenic		2.3 <2ppm	1	1	1	Test in on current supplier CofA		1	1	
	Magnesium stearate											
		environmental metal impurities		2.3 <20ppm	5	5	25	20 ppm only in JP		1	25	Test for environmental metals.
		Nickel		3915 <1ppm	5	1	5	USP and Ph.Eur 5 ppm. Identified as present in a raw material by supplier survey.	5 ppm	0.2	1	
		Cadmium		1.6 <1ppm	5	1	5	USP and Ph.Eur 3 ppm	3 ppm	0.2	1	
		Lead		1.6 <1ppm	5	1	5	USP and Ph.Eur 10 ppm. Specification 5 ppm from supplier survey.	5 ppm	0.2	1	
		As		2.3 <0.05 ppm	5	1	5	Periodic testing by ICP-OES confirmed by supplier survey.	1 ppm	0.2	1	
		Hg		23.5 <0.05 ppm	5	1	5	Periodic testing by ICP-OES confirmed by supplier survey.	1 ppm	0.2	1	
Coating	Film coating Hypromellose											
		environmental metal impurities		2.3 <20ppm heavy	5	5	25	USP, Ph.Eur and JP 20 ppm heavy metals	20 ppm	1	25	Test coating for environmental metals.
	Titanium Dioxide											
		environmental metal impurities		7.8 <20 ppm	10	5	50	USP, Ph.Eur and JP 20 ppm heavy metals	20 ppm	1	50	Test coating for environmental metals.
		Antimony		1873 <100ppm	10	1	10	Ph.Eur 100 ppm	100 ppm	0.2	2	
		Arsenic		2.3 <1ppm	10	1	10	USP 1ppm, Ph.Eur 5 ppm, JP 10 ppm	1ppm	0.2	2	
		Barium		28057	10	1	10	limit is absence by the test used. Unlikely to exceed allowed 2%		1	10	
		Iron		28057 <200ppm	10	1	10	Ph.Eur 200 ppm	200 ppm	0.2	2	
		Lead		156.6 <60 ppm	10	1	10	JP 60 ppm		1	10	
	Triacetin											
		environmental metal impurities			1	5	5			1	5	
	Blue #2											
		environmental metal impurities			10	5	50	No information available on this component. Testing of coating will inform risk-level.		1	50	Test coating for environmental metals.
		Al	N/A		10	0		Al colourant, low toxicological concern		1		
	Blue #1											
		environmental metal impurities			10	5	50	No information available on this component. Testing of coating will inform risk-level.		1	50	Test coating for environmental metals.
		Al	N/A		10	0		Al colourant, low toxicological concern		1		
Equipment	Equipment											
					10	1	10			1	10	
Packing	Primary Pack: PVC blisters											
		Potentially leached metals			1	1	1			1	1	
Process materials	Water											
		environmental metal impurities			1	5	5			1	5	

Figure 8.15 FMECA.

Table 8.7 Proposed testing.

Potential source of metal impurities	No. of batches to be analyzed	Elemental I impurities to include in analytical screening		Comments
		Environmental and naturally abundant elements	Intentionally added metals, for example, metal catalysts/ reagents	
Hypromellose	Three batches representative of the quality/supplier/grade to be used during commercial manufacture	Class 1: As, Cd, Hg, Pb Class 2A: V, Co, Ni	Sn	
Microcrystalline cellulose	Three batches		None	
Lactose monohydrate	Three batches		None	
Magnesium stearate	Three batches		None	
Crospovidone	None		None	Addressed through detailed supplier response
Coating	Three batches	Class 1: As, Cd, Hg, Pb Class 2A: V, Co, Ni		Aluminum lakes are used to color the coating blue
API	Three batches		Pd catalyst	

8.11 Overall Conclusions

At the time of writing this chapter ICH Q3D is entering its implementation phase for existing products. As it does so there remain a number of areas of uncertainty that need to be addressed, these include:

- Expectations for method validation: risk assessment screening versus routine QC testing
- Pharmacopoeial challenges: concern over differences between ICH Q3D and USP <232> and differences between USP <232> and Ph. Eur. 5.20 [35]. Harmonization between Q3D and <232> have reduced this concern. But it has not gone away
- Harmonization between Ph. Eur. 2.4.20 [36] and USP <233>
- Application of the "control threshold" (a new concept in Q3D, intended as a tool for risk assessment)

Also uncertainties exist in terms of regulatory expectations:

- Where should risk assessment appear in the common technical dossier (CTD)?
- What is expected in the risk assessment summary?
- Will expectations be consistent over time and across regions?
- How will risk assessments for existing products be conveyed to regulatory authorities?

In terms of components:

- What information should suppliers provide to their customers?
- How do we gain access to closed Drug Master File (DMFs) for established APIs?
- What if, for example, an excipient supplier changes the manufacturing process?

This list is not exhaustive.

Despite all of these concerns, it is important not to lose sight of the evidence to date regarding actual risk. The evidence, although at this stage not confirmatory, does not indicate a widespread risk. Testing, for example, of excipients has shown lower levels and less variance than possibly anticipated. Evidence also indicates that CCS presented a negligible risk.

It is hoped as ICH Q3D reaches maturity that risk assessments will take these factors into account and ultimately be predicated by true risk-based principles as opposed to being driven by extensive testing.

References

1 ICH Q3D. Guideline for Elemental Impurities, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3D/Q3D_Step_4.pdf. Accessed on April 12, 2017.

2 PF 21(6). November–December, 1995, p. 1632.
3 PF 29(4). July–August, 2003, p. 1328–1331.
4 Lewen N., Mathew M., Schenkenberger T., Raglione J. *J. Pharm. Biomed. Anal.*, 2004, 35, 739–752.
5 EMEA. Guideline on the Specification Limits for Residual Metal Catalysts for Metal Reagents, 2008, The docuemnt reference no: CPMP/SWP/QWP/4446/00 cor.
6 USP. Digest of Comments Received on the Stimuli Article "General Chapter on Inorganic Impurities: Heavy Metals" Published in Pharmacopeial Forum 34(5), April 22, 2009, http://www.usp.org/sites/default/files/usp_pdf/EN/USPNF/key-issues/2009-04-22MetalImpuritiesCommentDigest.pdf. Accessed on April 12, 2017.
7 ICH Q3D. Final Concept Paper Q3D: Impurities: Guideline for Elemental Impurities, July 17, 2009, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3D/Q3D_Final_Concept_Paper.pdf.
8 ICH Q3D. Implementation of Guideline for Elemental Impurities, document reference: CPMP/SWP/QWP/4446/00 corr.
9 ICH Q3C. Guideline for Residual Solvents, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3C/Step4/Q3C_R5_Step4.pdf. Accessed on April 12, 2017.
10 Teasdale A., Walsh P., Ulman K., Domoradzki J. *Pharm. Technol.*, 2015, 39, 9, 44–51.
11 EASE. Estimation and Assessment of Substance Exposure, HSE, 1999.
12 National Environmental Policy Institute. Assessing the Bioavailability of Metals in Soil for Use in Human Health Risk Assessments. Bioavailability Policy Project Phase II Metals Task Force Report, (Washington, DC, 2000).
13 ICH M7. Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M7/M7_Step_4.pdf. Accessed on April 12, 2017.
14 US Food & Drug Administration. Title 21—Food and Drugs Chapter I—Food and Drug Administration Department of Health and Human Services Subchapter C—Drugs: General Part 201—Labeling Subpart G—Specific Labeling Requirements for Specific Drug Products. Sec. 201.323 Aluminum in large and small volume parenterals used in total parenteral nutrition, https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=201.323. Accessed on April 12, 2017.
15 ICH Q9. Quality Risk Management, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q9/Step4/Q9_Guideline.pdf. Accessed on April 12, 2017.
16 ICH Q8. Pharmaceutical Development, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q8_R1/Step4/Q8_R2_Guideline.pdf. Accessed on April 12, 2017.

17 ICH Q11. Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/Biological Entities), http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q11/Q11_Step_4.pdf. Accessed on April 12, 2017.
18 Jones C.E., Taylor P.A., McEwan A.G., Hanson G.R. *J. Am. Chem. Soc.*, 2006, 128(29), 9378–9386.
19 Craig B., Anderson D., (Ed.), *Handbook of Corrosion Data*, ASM International, Materials Park, 1995.
20 WHO. *Guidelines for Drinking Water Quality*, WHO, Genève, 2011.
21 USP <1231>. Water for Pharmaceutical Purposes.
22 Bevilacqua A., Soli T.C. Stimuli to the Revision Process: Elemental Impurities in Pharmaceutical Waters, Pharmacopoeial Forum, 2013, 39(1).
23 Guidance for Industry. Container Closure Systems for Packaging Human Drugs and Biologics. Chemistry, Manufacturing, and Controls Documentation, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070551.pdf?_sm_au_=iJHnDqk055s57FQL. Accessed on April 12, 2017.
24 Jenke D.R., Stults C.L.M., Paskiet D.M., Ball D.J., Nagao L.M. *J. Pharm. Sci. Technol.*, 2015, 69, 1–48.
25 Markovic I. *Am. Pharm. Rev.*, 2009, 12(4), 96, 98–101.
26 Nashed-Samuel Y., Liu D., Fujimori K., Preez L., Lee H. *Am. Pharm. Rev.*, 2011, 14(1), 74, 76, 78–80.
27 Christensen S., Moellar E.H., Bonde C., Lilleoere A.M. *Eur. J. Pharm. Biopharm.*, 2007, 66(3), 366–371.
28 Liu W., Swift R., Torraca G., Nashed-Samuel Y., et al. *J. Pharm. Sci. Technol.*, 2010, 64(1), 11–19.
29 ICH Q2. Validation of Analytical Procedures: Text and Methodology, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf. Accessed on April 12, 2017.
30 USP <233>. Elemental Impurities Procedures.
31 Kauffman J., Ulman K.L., Schoneker D., Li G., Sturm J.J., Thackery L.M. *J. Pharm. Sci.*, 2015, 104(12), 4197–4206.
32 ICH Q10. Pharmaceutical Quality System, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q10/Step4/Q10_Guideline.pdf. Accessed on April 12, 2017.
33 Teasdale A., Chery C.C., Cook G., Glennon J., et al. *Pharm.Technol.*, 2015, 39(3), 36–49.
34 Holliday M.A., Segar W.E. *Pediatrics*, 1957, 19(5), 823–832.
35 European Pharmacopoeia supplement 9.2. Chapter 5.20: Metal Catalysts or Metal Reagent Residues.
36 European Pharmacopoeia supplement 9.2. Chapter 2.04.20: Determination of Metal Catalysts or Metal Reagent Residues.

9

ICH Q4

Pharmacopeial Harmonization and Evaluation and Recommendation of Pharmacopeial Texts for Use in the ICH Regions

David Elder

David P Elder Consultancy, Hertford, UK

9.1 Introduction

Following the successful implementation of ICH Q6A [1] and Q6B [2], the ICH steering committee recognized the need to drive harmonization of selected chapters/procedures across the three main pharmacopoeias (Ph. Eur., JP, and USP). This was in order to avoid any unnecessary testing by industry brought about through differences between regional pharmacopoeias. Further benefits that can be achieved through harmonization potentially include reduced costs and time to market and also simultaneous compliance with requirements of all three pharmacopoeias: a necessary requirement for submissions to the major markets. To illustrate this, Moore [3] used the carboxymethyl cellulose calcium monograph as a case study. Prior to the development of ICH Q4 [4], a total of 37 tests (USP = 13, JP = 13, Ph. Eur. = 11 tests) would be required for full compendial release in all three regions compared with a mere 10 tests post ICH Q4 finalization.

Given concerns over the lack of harmonization across the major pharmacopoeias, the industry requested the development of an ICH guideline to assist in harmonization, which resulted in the formation of an expert working group (EWG) to help address how the regulatory authorities from the three ICH regions (EMA, PMDA, and FDA) could ultimately interpret and mutually recognize the "interchangeability" of harmonized chapters/procedures. ICH Q4 [4] is split into Q4A (Pharmacopoeial Harmonization) and Q4B (Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions). The former guidance explains the working relationship between the Pharmacopoeial Discussion Group (PDG) and the EWG (the PDG consists of representatives from the European Directorate for the Quality of Medicines

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(EDQM), the PMDA, and the USP). In contrast, the latter guidance (ICH Q4B) forms the most significant part of the overall guideline. ICH Q4 and Q4B are often used interchangeably and this will also be the case in this chapter.

In November 2003, the EWG/PDG identified 11 general chapters (subsequently reduced to 10 with the combination of Uniformity of Content and Uniformity of Mass, as a rebranded Uniformity of Dosage Units) for complete harmonization. In November 2008, a further five new general chapters were added to the process. The implementation was via the ICH Q4B [4] EWG and the PDG. From the beginning, harmonization was always seen as challenging as the three pharmacopoeias represented very different constituencies, that is, governmental (JP), intergovernmental (Ph. Eur.), and nongovernmental (USP) organizations.

9.2 Overview of Guidelines

9.2.1 Harmonization Process

The harmonization process devised and outlined in ICH Q4B [4] is summarized in Figure 9.1. Harmonization can be performed either retrospectively for existing or prospectively for new monographs or chapters. The PDG working with experts from each pharmacopoeia produces the harmonized test for the various chapters/procedures via independent public consultation and then reports back to the EWG as appropriate. Public consultation can include meetings with industry and trade bodies, upon request.

The objective of harmonization is to develop interchangeable methods or requirements. The desired outcome for a general chapter is that the methodology in question will be designated as being harmonized across the chapters within the individual pharmacopoeias such that any result obtained using any one of the three general chapters would be considered equivalent. Similarly, when harmonization of monographs is performed, the objective is to develop identical requirements for all attributes of a product, that is, an excipient (total harmonization). However, it is recognized that in certain cases this can be very challenging due to differences in legal status or interpretation. In those cases, PDG decided to proceed with approval and publication of monographs, where as many attributes as possible were harmonized (harmonization by attributes). Where there are differences these are identified as non-harmonized attributes or, if a test is specific to an individual pharmacopoeia, as a local requirement.

Once finalized, it was agreed that no harmonized monograph/chapter would be unilaterally revised. To ensure effective control over changes, specific criteria governing change were defined. The following criteria for revision apply: (i) replacement of existing techniques with more accurate and more precise procedures, (ii) new methodology for preparation of reagents or products not

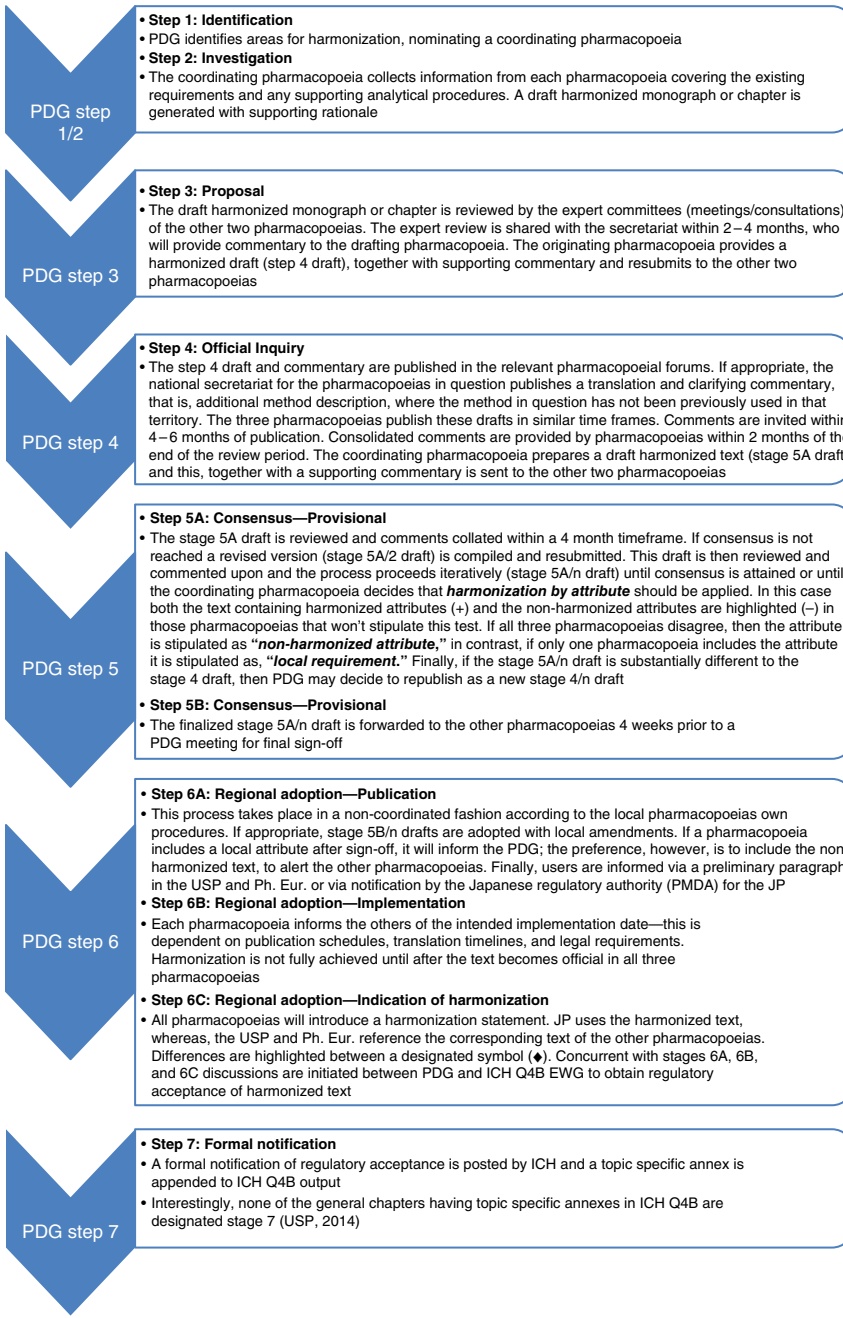

PDG step 1/2
• Step 1: Identification
• PDG identifies areas for harmonization, nominating a coordinating pharmacopoeia
• Step 2: Investigation
• The coordinating pharmacopoeia collects information from each pharmacopoeia covering the existing requirements and any supporting analytical procedures. A draft harmonized monograph or chapter is generated with supporting rationale
PDG step 3
• Step 3: Proposal
• The draft harmonized monograph or chapter is reviewed by the expert committees (meetings/consultations) of the other two pharmacopoeias. The expert review is shared with the secretariat within 2–4 months, who will provide commentary to the drafting pharmacopoeia. The originating pharmacopoeia provides a harmonized draft (step 4 draft), together with supporting commentary and resubmits to the other two pharmacopoeias
PDG step 4
• Step 4: Official Inquiry
• The step 4 draft and commentary are published in the relevant pharmacopoeial forums. If appropriate, the national secretariat for the pharmacopoeias in question publishes a translation and clarifying commentary, that is, additional method description, where the method in question has not been previously used in that territory. The three pharmacopoeias publish these drafts in similar time frames. Comments are invited within 4–6 months of publication. Consolidated comments are provided by pharmacopoeias within 2 months of the end of the review period. The coordinating pharmacopoeia prepares a draft harmonized text (stage 5A draft) and this, together with a supporting commentary is sent to the other two pharmacopoeias
PDG step 5
• Step 5A: Consensus—Provisional
• The stage 5A draft is reviewed and comments collated within a 4 month timeframe. If consensus is not reached a revised version (stage 5A/2 draft) is compiled and resubmitted. This draft is then reviewed and commented upon and the process proceeds iteratively (stage 5A/n draft) until consensus is attained or until the coordinating pharmacopoeia decides that harmonization by attribute should be applied. In this case both the text containing harmonized attributes (+) and the non-harmonized attributes are highlighted (–) in those pharmacopoeias that won't stipulate this test. If all three pharmacopoeias disagree, then the attribute is stipulated as "non-harmonized attribute," in contrast, if only one pharmacopoeia includes the attribute it is stipulated as, "local requirement." Finally, if the stage 5A/n draft is substantially different to the stage 4 draft, then PDG may decide to republish as a new stage 4/n draft
• Step 5B: Consensus—Provisional
• The finalized stage 5A/n draft is forwarded to the other pharmacopoeias 4 weeks prior to a PDG meeting for final sign-off
PDG step 6
• Step 6A: Regional adoption—Publication
• This process takes place in a non-coordinated fashion according to the local pharmacopoeias own procedures. If appropriate, stage 5B/n drafts are adopted with local amendments. If a pharmacopoeia includes a local attribute after sign-off, it will inform the PDG; the preference, however, is to include the non-harmonized text, to alert the other pharmacopoeias. Finally, users are informed via a preliminary paragraph in the USP and Ph. Eur. or via notification by the Japanese regulatory authority (PMDA) for the JP
• Step 6B: Regional adoption—Implementation
• Each pharmacopoeia informs the others of the intended implementation date—this is dependent on publication schedules, translation timelines, and legal requirements. Harmonization is not fully achieved until after the text becomes official in all three pharmacopoeias
• Step 6C: Regional adoption—Indication of harmonization
• All pharmacopoeias will introduce a harmonization statement. JP uses the harmonized text, whereas, the USP and Ph. Eur. reference the corresponding text of the other pharmacopoeias. Differences are highlighted between a designated symbol (♦). Concurrent with stages 6A, 6B, and 6C discussions are initiated between PDG and ICH Q4B EWG to obtain regulatory acceptance of harmonized text
PDG step 7
• Step 7: Formal notification
• A formal notification of regulatory acceptance is posted by ICH and a topic specific annex is appended to ICH Q4B output
• Interestingly, none of the general chapters having topic specific annexes in ICH Q4B are designated stage 7 (USP, 2014)

Figure 9.1 Pharmacopoeial harmonization process.

covered in existing monograph, (iii) unavailability of specified reagents or equipment, and (iv) health and safety considerations. Any revisions will be initiated by PDG, and a coordinating pharmacopoeia will be identified who will prepare a stage 3 draft, together with supporting commentary. Thereafter, the previously described harmonization process (stages 4–7) will be followed. An accelerated/abbreviated approach may be appropriate for health and safety considerations.

9.3 Initial Process

Using the process described, a series of general chapters were identified for harmonization. A summary of the status of the harmonized general chapters is provided in Table 9.1, and unless otherwise stipulated, the title of each of the regional pharmacopoeial monographs is as stated in the ICH Q4B [4] annex. Any differences in nomenclature are highlighted by superscripts with details in the appropriate comments section.

If there are no additional considerations listed in section 2 of the ICH Q4B [4] annex, then the interchangeability of the chapter has no preconditions. Otherwise text in section 2 of the annex provides guidance for the applicant on any specific regional requirements that will be expected as part of the regional implementation. Some of the general implementation considerations are summarized in Figure 9.2.

9.4 ICH Q4B Annexes

9.4.1 Overview/Summary

The test for Extractable Volume of Parenteral Preparations (Annex #2) was fully harmonized, and the acceptance criteria are the same in all three pharmacopoeia. Seven other tests were fully harmonized, but they contained the following additional commentary: "Can be used as interchangeable in the ICH regions. The proposed texts evaluated did not contain acceptance criteria." The seven tests were Microbiological Examination of Non-Sterile Products: Microbiological Enumeration Tests (#4A), Microbiological Examination of Non-Sterile Products: Tests for Specified Microorganisms (#4B), Microbiological Examination of Non-Sterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use (#4C), Polyacrylamide Gel Electrophoresis (#10), Capillary Electrophoresis (#11), Analytical Sieving (#12), and Bulk Density and Tapped Density of Powders (#13).

However, the remainder of the ICH Q4B annexes did have some specific implementation considerations, and these are summarized in Table 9.2.

Table 9.1 General chapters identified for harmonization.

General chapter	ICH Q4B annexes	Ph. Eur.	JP	USP	Comments
Residue on Ignition/Sulfated Ash	#1	2.4.14 (01/2007) [1]	2.44 [2]	<281> [2]	Sulfated ash [1]
					Residue on ignition [2]
Test for Extractable Volume of Parenteral Preparations	#2	2.9.17 (01/2006)	6.05	<1> [3]	The official text is incorporated into general chapter <1> of the USP. Injections, in section entitled, "Volume in Containers" [3]
Test for Particulate Matter Contamination: Subvisible Particles	#3	2.9.19 (01/2005) [4]	6.07 [5]	<788> [6]	Particulate contamination: subvisible particles [4]
					Insoluble particulate matter test for injection [5]
					Particulate matter in injection [6]
Microbiological Examination of Non-Sterile Products: Microbiological Enumeration Tests	#4A	2.6.12 (01/2009)	4.05	<61>	
Microbiological Examination of Non-Sterile Products: Tests for Specified Microorganisms	#4B	2.6.13 (01/2009)	4.05	<62>	
Microbiological Examination of Non-Sterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use	#4C	5.1.4 (01/2009) [7]	12 [8]	<1111> [8]	Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use [7]
					Microbiological attributes of non-sterile pharmaceutical products [8]
Disintegration Test	#5	2.9.1 (01/2009)	6.09	<701>	
Dissolution Test	#6	2.9.3 (01/2010) [9]	6.10 (see Section 9.5.4)	<711> [10]	Dissolution test for solid dosage forms [9]
					Dissolution [10]

(*Continued*)

Table 9.1 (Continued)

General chapter	ICH Q4B annexes	Ph. Eur.	JP	USP	Comments
Uniformity of Dosage Units	#7	2.9.40 (04/2012) [11] (see Section 9.5.5)	6.02 [12]	<905> [13]	First issued 04/2008, updated 04/2012 [11]
					First issued 11/2008, updated 05/2013 [12]
					First issued 10/2010, updated 12/2011 [13]
Sterility Test	#8	2.6.1 (01/2009) [14]	4.06	<71>	Sterility [14]
Tablet Friability	#9	2.9.7 (01/2010) [15]	26	<1216> [16]	Friability of uncoated tablets [15]
					Tablet friability [16]
Polyacrylamide Gel Electrophoresis	#10	2.2.31 (01/2008)	23 [17]	<1056> [18]	SDS-polyacrylamide gel electrophoresis [17]
					Biotechnology derived articles: polyacrylamide gel electrophoresis [18]
Capillary Electrophoresis	#11	2.2.47 (01/2008)	4	<1053> [19]	Biotechnology derived articles: capillary electrophoresis [19]
Analytical Sieving	#12	2.9.38 (07/2008) [20]	3.04 [21]	<786> [20]	Particle size distribution estimation by analytical sieving [20]
					Particle size determination [21]
Bulk Density and Tapped Density of Powders	#13	2.9.34 (07/2010)	3.01 [22]	<616>	Determination of bulk and tapped densities [22]
Bacterial Endotoxins Test	#14	2.6.14 (01/2010) [22]	4.01	<85>	Bacterial endotoxins [22]
Color [23]	#15	NA	NA	NA	Not officially harmonized as yet [23]

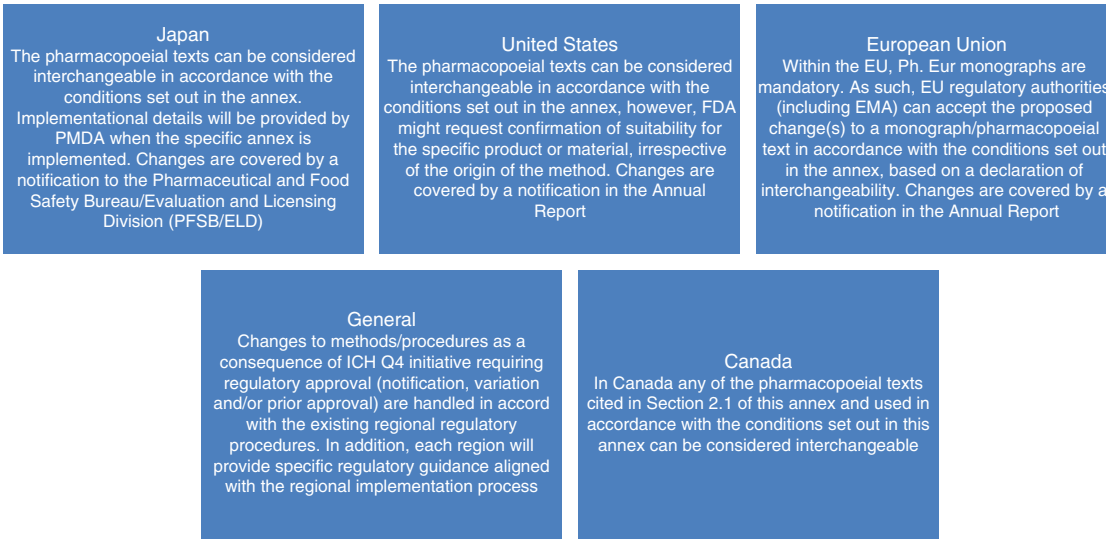

Japan
The pharmacopoeial texts can be considered interchangeable in accordance with the conditions set out in the annex. Implementational details will be provided by PMDA when the specific annex is implemented. Changes are covered by a notification to the Pharmaceutical and Food Safety Bureau/Evaluation and Licensing Division (PFSB/ELD)
United States
The pharmacopoeial texts can be considered interchangeable in accordance with the conditions set out in the annex, however, FDA might request confirmation of suitability for the specific product or material, irrespective of the origin of the method. Changes are covered by a notification in the Annual Report
European Union
Within the EU, Ph. Eur monographs are mandatory. As such, EU regulatory authorities (including EMA) can accept the proposed change(s) to a monograph/pharmacopoeial text in accordance with the conditions set out in the annex, based on a declaration of interchangeability. Changes are covered by a notification in the Annual Report
General
Changes to methods/procedures as a consequence of ICH Q4 initiative requiring regulatory approval (notification, variation and/or prior approval) are handled in accord with the existing regional regulatory procedures. In addition, each region will provide specific regulatory guidance aligned with the regional implementation process
Canada
In Canada any of the pharmacopoeial texts cited in Section 2.1 of this annex and used in accordance with the conditions set out in this annex can be considered interchangeable

Figure 9.2 ICH Q4 implementation considerations.

Table 9.2 General chapters with some specific implementation guidelines.

General chapter	ICH Q4B annexes	Comments
Residue on Ignition/Sulfated Ash	#1	An appropriately sized sample (ca. 1–2 g) is required to ensure that the residual amount left after ashing is >1 mg and hence can be accurately measured gravimetrically. The sample size/acceptance criteria need to be appropriate justified in the dossier. The muffle furnace requires appropriate calibration to align with regional GMP compliance requirements
Test for Particulate Matter Contamination: Subvisible Particles	#3	Except for parenteral products with a nominal volume of ≤100 ml the test is interchangeable. The JP criteria are more stringent than that of Ph. Eur. or USP for the ≤100 ml volume and are therefore not harmonized. The instrument requires appropriate calibration and system suitability measurements to align with regional GMP compliance requirements
Disintegration Test	#5	For larger solid oral dosage forms (>18 mm) in length, there is a different non-harmonized procedure. For solid oral dosage forms with a functional coat, for example, delayed release, gastro-resistant, or enteric coated, the test is non-harmonized. Any product-specific parameters, for example, media, use of discs, etc., need to be appropriately justified in the dossier. In addition, specification acceptance criteria are out with the scope of the monograph and need to be appropriately justified in the dossier
Dissolution Test	#6	The monographs for Apparatus 1 (basket), Apparatus 2 (paddle), and the flow through apparatus are considered interchangeable—the latter apparatus should be unambiguously described in the submission as the numbering is not harmonized across the different pharmacopoeias
		However, there are a significant number of caveats to interchangeability. The test is not harmonized if enzymes are used in the media, nor for large vessels (>1 l). Like disintegration, the test is not harmonized for solid oral dosage forms with a functional coat (see earlier). Appropriate calibration and system suitability measurements to align with regional GMP compliance requirements are required. Additional validation studies are required to assess the impact of probes (temperature monitoring devices are specifically mentioned, but this is equally germane to sampling probes) on the test data. The test is not interchangeable for JP interpretation 2. In addition, specification acceptance criteria are out with the scope of the monograph and need to be appropriate justified in the dossier. Any product-specific parameters, for example, apparatus type, stirring speed, media, use of sinkers, etc., needs to be appropriately justified in the dossier

Table 9.2 (Continued)

General chapter	ICH Q4B annexes	Comments
Uniformity of Dosage Units	#7	Unless the tablet weight (>25 mg) or API loading (>25% by weight) criteria are met, then the use of the MV/WV variants of the test is not appropriate. Interchangeability is not applicable where specific dosage forms in regional pharmacopoeias have additional text enclosed by black diamond symbols. If a correction factor is required when different procedures are used for assay and content uniformity, then this factor needs to be appropriately justified in the dossier
Sterility Test	#8	From an interchangeability perspective, the dilution and rinsing fluids should have no intrinsic antimicrobial properties. For liquid preparations with a nominal volume of ≤100 ml in batches of >500 containers, the test is interchangeable if the sample size is >2% or 20 containers, whichever is smaller. The testing requirements for medical devices, for example, sutures, are out with the guidance
Tablet Friability	#9	From an interchangeability perspective, the mass loss from three determinations should be not more than 1.0%, unless specified in the individual dossier
Bacterial Endotoxins Test	#14	Any of the pharmacopoeial techniques can be utilized.[a] In the event of uncertainty or dispute, the gel–clot (LAL) limit test should be utilized. To make decisions on product utility, the reference standards from the three pharmacopoeias are considered interchangeable as they have all been externally calibrated against the WHO international standards for endotoxins. Applicants should perform replicate tests (at least in duplicate) on solutions A–D, using the optimal processes as recommended by the reagent supplier

[a] LAL, limulus amoebocyte lysate; MATPT, monocyte activation type pyrogen test; RHCFC, recombinant horseshoe crab factor C assay.

9.5 Commentary on ICH Q4B Annexes with Specific Implementation Guidance

9.5.1 Residue on Ignition/Sulfated Ash (Annex 2, #1)

The current pharmacopoeial procedures to control inorganic impurities (see ICH Q3D [5]) include the Heavy Metals test (non-harmonized—current stage 3), allied with the Residue on Ignition/Sulfated Ash test (harmonized: Annex 2, #1), together with other wet-chemistry tests. Both USP and Ph. Eur. will remove the existing Heavy Metals monograph requirements when they implement the

harmonized ICH Q3D guidelines. It is not currently clear at the time of writing what approach the JP will take. The USP will introduce new general chapters for the testing methodologies (<232>) and limits (<233>) to be implemented in January 2018. Similarly, Ph. Eur. will harmonize their requirements with ICH Q3D and will implement in December 2017 [6].

9.5.2 Test for Particulate Matter contamination: Subvisible Particles (Annex 2, #3)

There is ongoing discussion as to whether this harmonized test is actually fit for purpose, particularly with respect to biopharmaceutical parenteral products. For these biological products there are concerns that particles formed by aggregation may be the cause of many undesirable immunogenic responses. The light obscuration test is challenging when applied to certain types of formulation, for example, those with high viscosity or turbidity, or formulations that readily aerate causing bubble formation—leading to erroneously high particle counts or where the particles may appear semitransparent, for example, protein particulates—leading to inaccurate particle counting. In addition, although this test centers on the number of allowable particulates, there should be consideration on the type of particulates as well—as not all particulates are equal with respect to safety considerations [7]. It is interesting that ICH EWG/PDG chose not to harmonize the general chapters for visible particles (USP <790>, Ph. Eur. 2.9.20, JP 6.06) at the same time that subvisible particles were being reviewed and harmonized (the inspection conditions have been harmonized between USP and Ph. Eur.).

9.5.3 Disintegration Test (Annex 2, #5)

A particular challenge when attempting to harmonize procedures was the slightly different technical specifications for this test across the three pharmacopoeias. ICH Q4B [4] circumvented this issue by stating that the specific method criteria would be out of scope (i.e., any product-specific parameters, e.g., media, use of discs, etc., are appropriately justified in the dossier).The test is fully harmonized except for disintegration of solid oral dosage forms with a functional coat, for example, delayed release and gastro-resistant or enteric-coated tablets/capsules (JP exceptions). These are a two-stage test, with the first stage assessing gastro-resistance, that is, the dosage form should not disintegrate in acidic media, whereas the second stage is a classical disintegration test in a media of appropriate pH. It is likely, given JP's concerns regarding the relevance of a test in acidic media (with the high prevalence of achlorhydria among the elderly Japanese population), that the JP felt that this test could not be harmonized. In addition, for larger solid oral dosage forms (>18 mm) in length, there is a different non-harmonized procedure. Other non-harmonized attributes include exclusion of granules, dry syrups, pills (JP), exclusion of an

auxiliary tube (JP), and exclusion of water as immersion fluid as the default approach. All of these non-harmonized attributes reflect the differing approach within Japan toward *in vitro* testing of dosage forms.

9.5.4 Dissolution Test (Annex 2, #6)

Harmonization of dissolution testing (Annex 2, #6) was perhaps one of the greatest challenges within the ICH Q4 EWG/DPG initiative.

As with disintegration testing, the general chapter covering dissolution of solid oral dosage forms with a functional coat, for example, delayed release and gastro-resistant or enteric-coated tablets/capsules, could not be harmonized (JP exceptions). Areas of concern include

- Non-harmonization of Apparatus 3 (reciprocating cylinder).
- Vessels larger than 1 l.
- Pooling of samples.
- JP interpretation 2 (i.e., if individual dissolution rates of not less than 10/12 dosage units meets the requirements).
- If enzymes are used in the dissolution media. This is proving to be an ongoing area of discord with the USP recently proposing to introduce enzymes into the test and EDQM (European Directorate for the Quality of Medicines) again rejecting the proposal for a harmonized monograph.

Given the inherent challenges of trying to harmonize dissolution testing, it is perhaps unsurprising that no attempt was made to improve/enhance the most common dissolution apparatus, that is, Apparatus 1 (rotating basket) and Apparatus 2 (rotating paddle). For example, the rotating paddle method is poorly designed from an efficient mixing and hydrodynamics perspective and demonstrates high levels of intrinsic variability. Several international collaborative studies with USP calibrator tablets have confirmed these findings [8, 9]. In the latter study [9], over half of the participating laboratories (15/28) produced failing results for USP calibrator tablets.

A recent technical report [10] submitted to FDA on the effect of hydrodynamics in the apparatus using computational fluid dynamic (CFD) simulations of the dissolution velocity profiles showed that significant differences exist in terms of both flow rate and shear rate at different locations near the bottom of the vessel and that significantly these differences occur over very small spatial distances. In particular, there is a "near-stagnant region" that exists immediately underneath the paddle shaft. This means that the exact location of the tablet/capsule is critical to product performance, and given the inherent randomness of the settling process of the dosage form as it is added to the vessel, these factors introduce significant uncertainty into the test results. That is, the apparatus is poorly designed for its intended purpose, which is not surprising considering that it consists of a small, hemispherical vessel, without baffles, but

with a slowly rotating paddle (to make it more discriminating), into which a dosage form is randomly dropped. They commented that such a test would lead to both type I (release of defective product) and type II (rejection of good product) errors. The authors made several important recommendations:

- Recognize that dissolution testing, in its current embodiment, is based on a poor understanding of the relevant, underpinning physical principles of dynamic mixing.
- Discontinue the practice of trying to preassign variability to the test by using calibrator tablets. It needs to be recognized that product and test variability cannot be decoupled.
- Discontinue the practice of trying to calibrate dissolution equipment. As the equipment is intrinsically simple, there should be no requirement to calibrate.
- Accept criteria based on total variability.
- Initiate studies to better understand *in vitro* release from solid oral dosage forms based on first principles, and use this enhanced understanding to develop a better test.

There have been several strategies over the years that aimed at addressing these fundamental design flaws of the dissolution apparatus, among them are peak vessels [11], crescent-shaped spindles [12], metal strips [13], and permanent in-line probes—acting as baffles [14] and the off-center paddle [15]. However, none of these approaches have gained widespread support across the industry, academia, or regulators.

9.5.5 Uniformity of Dosage Units (Annex 2, #7)

Uniformity of dosage units still continues to cause significant disagreements between the pharmacopoeias, particularly the alternative weight variation (WV) option. USP does not agree with the other pharmacopoeias that for solid oral dosage forms that do not meet the 25 mg/25% threshold limit, they can be tested using WV instead of content uniformity, if the variability of the data is less than, or equal to 2% RSD. In addition, content uniformity criteria for large sample sizes ($n = 100–10{,}000$), which can be generated when using process analytical testing (PAT) tools, for example, near-infrared spectroscopy (NIR), are often inappropriately restrictive. The traditional acceptance criteria for a standard sample size ($n = 20$), based on the allowable number of outliers in the 85–115% range and in particular no allowable outliers in the 75–125% range, are too strict where the sample size approaches that of the entire population. Ph. Eur. has proposed an additional test for uniformity of dosage units for large sample sizes (2.9.47), which uses two equivalent statistical approaches (I (parametric test) and II (nonparametric test)). Both of these alternative tests (I and II) use the same statistical concepts as the existing test [16, 17]. A new general chapter has been proposed.

In addition, many APIs exhibit some degree of asymmetry in their particle size distributions and the impact of this "skewed distribution" on content uniformity has been modeled [18]. The simulations show a high risk of overpotency, with low potency compounds showing a positively skewed distribution. This risk can be mitigated by either sieving (removing over sized particles) or size reduction (milling or micronization).

In a similar vein, Lunney and Anderson [19] used modeling to assess the predictivity of the harmonized content uniformity test. They found that the test was relatively insensitive to detect nonconforming products. This was attributed to the sample size for stage I testing (10 units), which is too small to give a reasonable estimation of the population standard deviation. The authors observed that a failure at stage I always resulted in an additional opportunity to pass at stage II testing. They reflected that considering the insensitivity of stage I, a more reasonable proposition would be to stop testing after a failure at stage I. However, their biggest concern was the insensitivity of the test at stage II (30 units), and this was assigned to the fact that the calculated tolerance intervals are too broad. They did comment that the test did perform consistently and robustly (cf. earlier version), with respect to underlying product distribution, but that it was overly sensitive to an underlying assumption of normality of data, that is, the data showed a standard normal distribution rather than a skewed distribution as is typically the case [18].

9.5.6 Sterility Test (Annex 2, #8)

Again there is only partial harmonization of the sterility test. Rinsing/diluting fluids were not harmonized, neither was there an agreement of the media for antibiotic preparations, for example, penicillins and cephalosporins. Alternative organisms to the established *Pseudomonas aeruginosa* (i.e., *Kocuria rhizophila*) or *Clostridium sporogenes* (i.e., *Bacteroides vulgatus*) were not agreed. Finally, there was a disagreement on the requirements for prefilled syringes, antibiotic solids for injections, antibiotic solids, bulks and blends, sterile aerosol products, and devices with pathways labeled sterile. The observation and interpretation of the results were however fully agreed.

9.5.7 Tablet Friability Test (Annex 2, #9)

The friability test for compressed tablets (cores or coated) is a very simple test using a very straightforward apparatus (rotating drum with internal baffle) that exposes tablets to both gravitational and frictional forces [20]. The loss of weight that occurs for a fixed sample weight (ca. 6.5 g) of the tablets, during a fixed number of revolutions (100×) of a cylindrical drum rotating at a fixed speed, provides an intrinsic measure of the likely damage that could be caused by abrasion/attrition that tablets can encounter during further processing stages (film coating of tablet cores) or during packaging and distribution (film

coated tablets). The test is performed in triplicate and there is a default limit of <1% (unless otherwise justified in the dossier). In addition, if there are any obviously cracked, cleaved, or broken tablets present in the tablet sample after the test, the sample fails. Because of its intrinsic simplicity, harmonization of this test/apparatus was very straightforward and there are no local rules in any of the pharmacopoeias. However, the standard pharmacopoeial test is not very good at predicting delamination of bi- or tri-layer tablets during film coating or distribution. For these types of dosage forms, more robust friabilators are often recommended.

9.5.8 Bacterial Endotoxins Test (Annex 2, #14)

FDA recently provided additional guidance on pyrogen and endotoxin testing [21] indicating that "because the compendial chapters and standards do not address certain regulatory perspectives, FDA is providing supplemental information in this guidance to explain our current thinking regarding the submission and maintenance of pyrogen and endotoxins testing for FDA-regulated products." One aspect of the additional guidance is the effect of quality by design (QbD) on endotoxin assessment. FDA indicated that "the strategy for endotoxins testing should be based upon product and process understanding in combination with risk management to ensure consistent final product quality. The appropriate in-process testing should be used to evaluate the production process areas at risk of endotoxins formation or incursion. Many firms already have programs for monitoring incoming ingredients and components, including the processing water, for endotoxins contamination. The finished product release specification should be considered when determining in-process limits for each phase of manufacturing tested. For purposes of evaluating the relative risk of product contamination, quantitative testing may be preferable to limit testing to facilitate product quality trending and to identify and correct excursions before they exceed the specification and cause product failure. An endotoxins limit should be justified on a case-by-case basis, and will be evaluated as a part of each relevant marketing application or supplement." At the current time it isn't clear what the effect of these additional requirements will have on the harmonized status of the bacterial endotoxin test (Annex 2, #14).

9.6 Pharmacopoeial Harmonization Post-ICH Q4

In November 2010, ICH Q4B EWG was disbanded by the ICH Steering Committee as all of the chapters within its scope were essentially completed. PDG committed to notify the ICH Secretariat of any future revisions to chapters previously harmonized under ICHQ4B [4]. Post 2011, all pharmacopoeial

harmonization has been carried out under the auspices of the pharmacopoeias/PDG. This section will detail progress made post 2011 [22]. Over this period PDG activities have focused on the continuing harmonization of general chapters along with an additional focus on excipient monographs. The outcomes of these activities will now be considered.

9.6.1 Ongoing Harmonization of Pharmacopoeial General Chapters

As of 2014, 23 out of 35 general chapters that were under consideration were harmonized (stage 5 or beyond, where stage 5 is defined as consensus), including all of the monographs identified during the original ICH process. Interestingly, none of the general chapters had reached stage 7—inter-regional acceptance. Both USP and Ph. Eur. had general chapters covering the status of harmonization (USP <1196>, Ph. Eur. 5.8 (01/2014)), but subsequently USP stopped updating USP <1196> preferring a web-based process (see Section 9.6.2). Comparison of these two general chapters is revealing. While USP <1196> identified 22 general chapters as having reached stage 6 (regional adoption and implementation), Ph. Eur. 5.8 only identified 18 out of 22 general chapters as being harmonized and omitted some of the most important general chapters (dissolution and uniformity of dosage units), as well as microbial contamination and protein determination. The reasons for this disconnect are not clear.

9.6.2 Current Harmonization Status of Pharmacopoeial Excipient Monographs

In addition to harmonization of general chapters, harmonization of a series of common excipients was also undertaken. As of 2014, 34 out of 61 excipient monographs that were under consideration were harmonized (stage 5 or beyond, where stage 5 is defined as consensus). Again, comparison of the USP and Ph. Eur. general chapters on harmonization is revealing. While USP <1196> identifies 31 specific monographs as having reached stage 6 (regional adoption and implementation), Ph. Eur. 5.8 only identifies 6 out of 31 excipient monographs as being harmonized. USP has recognized the difficulties inherent in keeping harmonization activities current and as of December 1, 2014 they discontinued USP <1196>. USP indicated that the reasons were increased transparency of harmonization process to key stakeholders. USP has three edition types of the pharmacopoeia (print, online, and flash drive) and as all three need to be identical; when a change occurs midway through the publication cycle, USP needs to publish this on their public website (http://www.usp.org/). In order to make the harmonization process more transparent, all harmonization-related topics will be published on the USP public website (http://www.usp.org/usp-nf/harmonization). From the website, the reader can get the information about the PDG history, purpose, process and working procedure, etc. One of the important topics is the "Notices of Stage 6 Harmonized Text"

(http://www.usp.org/usp-nf/official-text/stage-6) on which the most recently PDG signed-off monographs or general chapters have been posted. This website will allow USP to revise the status quicker than using the general chapter, allowing stakeholders to be aware of updates to the monographs and general chapters associated with harmonization (RH. Lafaver, personal communication with USP, September 29, 2014).

Reviewing the individual monographs identified at stage 6 (regional harmonization), the biggest impediment to full alignment appears to be the heavy metal test (linked with ICH Q3D [5]). This will be addressed under ICH Q3D [5], and there is a commitment from USP and Ph. Eur. to align with ICH Q3D, to remove the general chapter for heavy metals test and to introduce new tests/test limits aligned in late 2017/early 2018. JP's position still remains unclear at the time of writing.

Some parts of the microbial general chapters (microbial enumeration tests <61>; ICH Q4B #4A and tests for specified microorganisms <62>; ICH Q4B #4B) are also influencing ongoing harmonization of some excipients of natural origins, for example, starch.

9.7 Other Initiatives

In parallel, with the ICH/PDG harmonization initiatives, the three pharmacopoeias are all involved in other collaborative exercises, for example, USP collaboration with Chinese Pharmacopoeia Commission (ChP) and British Pharmacopoeia (BP), USP monograph modernization initiative [23], etc. In parallel, pharmacopoeias receive requests from their own regulatory bodies asking them to address certain perceived issues, for example, FDA modernization request to USP, EMA request to EDQM about quality of water for injection [24], and EC/EMA request to EDQM to establish an annual program of inspections of foreign API suppliers [25].

9.8 Conclusions

Unfortunately, the ICH Q4 guidelines are generally seen as one of the least successful of the ICH quality initiatives. However, this is probably unduly judgmental. It was recognized that pharmacopoeial harmonization of general chapters and specific monographs was always going to be challenging; however, the progress at times has been extremely slow. The main reason is that the 7-stage pharmacopoeial harmonization process (see Figure 9.1) is a long and iterative process and very open to being side tracked, with individual/regional perspectives guaranteed to take long periods of time to resolve. Some of the challenges were differences in legal status, or interpretation, or cultural differences, which were always likely to arise given the very different status of

pharmacopoeias that were involved: USP (nongovernmental), JP (governmental), and Ph. Eur. (intergovernmental). Very few, if any of the general chapters that reached stage 6 agreement (regional consensus), are totally harmonized (as per the definition)—most have been harmonized by attributes and there still appears "black diamond" text in these monographs reflecting regional differences.

Although the primary reason for harmonization, that is, supporting the implementation of the ICH Q6A [1]/Q6B [2] guidelines was achieved, the lack of meaningful harmonization has still resulted in extra testing by industry, which is non-value additive. In addition, the goal of pharmacopoeial harmonization to support global submissions appears to be even less likely than at the start of the ICH Q4 process. The existing, PDG-driven pharmacopoeial harmonization initiative only meaningfully supports US, EU, and Japanese submissions. China, India, Brazil, Argentina etc., now all expect compliance with their national pharmacopoeias. China in particular has very different requirements to that outlined in the PDG/ICH initiatives.

FDA has also indicated in the general guidance to ICH Q4B [4] guidelines that they may request confirmation of suitability of the harmonized guidance for the specific product or material, irrespective of the origin of the method. This is reflected in "black diamond" text in all general chapters. As a result, it cannot be claimed that this initiative has meaningfully reduced costs or time to market.

As of 2014, only two-thirds (23 out of 35) of the general chapters, and just over half (34 out of 61) of the specific monographs that were under consideration, were meaningfully harmonized (stage 5 or beyond, where stage 5 is defined as consensus). ICH [26] has reflected that "Even for general chapters that are being considered by PDG for harmonisation, the fundamental approaches taken by the various pharmacopoeias are not always consistent, making the likelihood of a harmonised outcome unlikely."

The pharmacopoeial harmonization initiative (post 2011) is different from the original ICH Q4 process: the PDG is now working independently of ICH EWGs and the process is being managed by the pharmacopoeias EWGs. Both USP and Ph. Eur. had general chapters covering the status of harmonization (USP <1196>, Ph. Eur. 5.8 (01/2014)); however, USP <1196> has recently been withdrawn, and USP now utilizes "Notices of Stage 6 Harmonized Text" (http://www.usp.org/usp-nf/official-text/stage-6) to communicate the progress of harmonization activities. The principal impediment to total harmonization of excipient monographs often appears to be the heavy metal test, but this is being addressed as part of the ICH Q3D [5] implementation initiative.

The ICH Q4 website is no longer being updated with the current status of the ongoing harmonization initiatives, and no general chapters or specific monographs have reached stage 7 (interregional acceptance, aligned with ICH Q4B [4]). Indeed, harmonization now appears to be "agreed" as stage-6 acceptance (regional acceptance)—see Tables 9.3 and 9.4 for overall status of

Table 9.3 Current harmonization status of pharmacopoeial general chapters (USP, 2016).

General chapter (revisions are designated in brackets)	Harmonization stage[a]	Coordinating pharmacopoeia	Comments
Amino Acid Determination	6	USP	PDG initiative (out with ICH Q4B)
Bacterial Endotoxins (R2)	4	JP	ICH Q4B, #14. PDG sign off on 16 June 2011
Biotechnology Derived Articles: Polyacrylamide Gel Electrophoresis (R1)	6	Ph. Eur.	PDG initiative (out with ICH Q4B). PDG sign off on 26 June 2014
Bulk Density and Tapped Density (R3)	6	Ph. Eur.	ICH Q4B, #13. PDG sign off on 6 November 2013
Conductivity	2	Ph. Eur.	PDG initiative (out with ICH Q4B)
Color (Instrumental Method)	3	Ph. Eur.	Part of initial ICH Q4B initiative (#15), but no substantive progress
Density of Solids	5B	Ph. Eur.	PDG initiative (out with ICH Q4B)
Disintegration	6	USP	ICH Q4B, #5
Dissolution (R3)	6	USP	ICH Q4B, #6. PDG sign off on 10 June 2010
Capillary Electrophoresis	6	Ph. Eur.	ICH Q4B, #11
Polyacrylamide Gel Electrophoresis	6	Ph. Eur.	ICH Q4B, #10
Extractable Volume	6	Ph. Eur.	ICH Q4B, #2—revised
Heavy Metals	3	USP	PDG initiative (out with ICH Q4B)
Inhalation	4	Ph. Eur.	PDG initiative (out with ICH Q4B)
Isoelectric Focusing	6	Ph. Eur.	PDG initiative (out with ICH Q4B)
Laser Diffraction Measure of Particle Size	4	Ph. Eur.	PDG initiative (out with ICH Q4B)

Table 9.3 (Continued)

General chapter (revisions are designated in brackets)	Harmonization stage[a]	Coordinating pharmacopoeia	Comments
Microbiological Examination of Non-Sterile Products: Microbiological Enumeration Tests	6	Ph. Eur.	ICH Q4B, #4A
Microbiological Examination of Non-Sterile Products: Tests for Specified Micro-organisms	6	Ph. Eur.	ICH Q4B, #4B
Microbiological Examination of Non-Sterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use	6	Ph. Eur.	ICH Q4B, #4C
Optical Microscopy	6	USP	PDG initiative (out with ICH Q4B)
Analytical Sieving (R1)	5B	USP	ICH Q4B, #12. Currently being revised
Test for Particulate Matter Contamination: Subvisible Particles (R1)	6	Ph. Eur.	ICH Q4B, #3—revised
Peptide Mapping	6	USP	PDG initiative (out with ICH Q4B)
Porosimetry by Mercury Intrusion	4	Ph. Eur.	PDG initiative (out with ICH Q4B). PDG sign off on 8 May 2007. JP will not implement this chapter
Powder Fineness	6	USP	PDG initiative (out with ICH Q4B). PDG sign off on 8 May 2007
Powder Flow	6	USP	PDG initiative (out with ICH Q4B)

(*Continued*)

Table 9.3 (Continued)

General chapter (revisions are designated in brackets)	Harmonization stage[a]	Coordinating pharmacopoeia	Comments
Protein Determination	6	USP	PDG initiative (out with ICH Q4B)
Residue on Ignition/ Sulfated Ash (R2)	6	JP	ICH Q4B, #1—revised
Specific Surface Area	6	Ph. Eur.	PDG initiative (out with ICH Q4B)
Sterility Test	6	Ph. Eur.	ICH Q4B, #8
Tablet Friability	6	USP	ICH Q4B, #9
Thermal Behavior of Powder	3	Ph. Eur.	PDG initiative (out with ICH Q4B)
Uniformity of Dosage Units (R1)	6	USP	ICH Q4B, #7. PDG sign off on 9 November 2010
Uniformity of Delivered Dose of Inhalations	2	Ph. Eur.	PDG initiative (out with ICH Q4B)
Water–Solid Interaction	3	Ph. Eur.	PDG initiative (out with ICH Q4B)
X-Ray Powder Diffraction	4	Ph. Eur.	PDG initiative (out with ICH Q4B)

Accurate at the time of writing, that is, 2Q2016.
[a] See Figure 9.1

harmonization initiative. Finally, some of the very real opportunities presented by the ICH Q4 [4] initiative were not meaningfully grasped, in particular, the harmonization of the technical aspects of some key underpinning analytical apparatus. The most obvious example of this is the dissolution apparatus. Dissolution is now being viewed as the cornerstone of ICH QbD initiatives and the setting of clinically relevant specifications [27]. Thus in the current paradigm, dissolution testing must be both clinically predictive and, at the same time, a robust and reproducible QC tool [27]. Given the technical inadequacies of the current dissolution apparatus (particularly the rotating paddle (Apparatus 2)), which are acknowledged, even by the pharmacopoeias and regulatory agencies, as providing very poor hydrodynamic mixing (especially at early time points, i.e., <30 min), it was a missed opportunity that the pharmacopoeias did not attempt to introduce a modified rotating paddle apparatus as part of the harmonization process that could address these key technical concerns. Substantive alternative proposals [11–15] have been made in the scientific literature, but these suggestions have never gained any real traction within the harmonization process.

Table 9.4 Current harmonization status of pharmacopoeial excipient monographs (USP, 2016).

Excipient monographs (revisions are designated in brackets)	Harmonization stage[a]	Coordinating pharmacopoeia	Comments[b]
Alcohol (R2 corr. 2)	6	Ph. Eur.	Harmonized by attributes, apart from description. Local requirements for identification, C and D (Ph. Eur.), list of impurities (Ph. Eur.), and expiration date (JP). PDG sign off on 26 June 2014
Benzyl Alcohol (R2)	6	Ph. Eur.	Harmonized by attributes, apart from appearance of solution. PDG sign off on 3 June 2008
Dehydrated Alcohol (R2 corr. 2)	4	Ph. Eur.	As for Alcohol. PDG sign off on 26 June 2014
Butyl Paraben (R1)	6	Ph. Eur.	Harmonized by attributes. Local requirements are heavy metals—20 ppm (JP). PDG sign off on 9 June 2010
Calcium Carbonate	4	USP	No commentary on harmonization in existing USP37/NF31 monograph
Calcium Disodium Edetate	6	JP	Harmonized by attributes. Non-harmonized attributes are clarity and/or color of solution, heavy metals, identification by IR absorption, and limit of nitrilotriacetic acid. Local requirements are for description (JP) and residual iron (Ph. Eur.)
Calcium Phosphate Dibasic (and Anhydrous)	6	JP	Harmonized by attributes. Non-harmonized attributes are heavy metals, fluoride, and iron limit tests
Carmellose Calcium (R1)	6	USP	See "Carmellose"
Carmellose Sodium	4	USP	See "Carmellose"
Carmellose	4[c]	JP	Harmonized by attributes. Non-harmonized attributes are heavy metals

(*Continued*)

Table 9.4 (Continued)

Excipient monographs (revisions are designated in brackets)	Harmonization stage[a]	Coordinating pharmacopoeia	Comments[b]
Cellulose Acetate (R2)	6	USP	Harmonized by attributes, apart from heavy metals test. This monograph is not included in JP that did not participate in harmonization exercise. PDG sign off on 7 November 2012[d]
Cellulose Acetate Phthalate (R1)	6	USP	Harmonized by attributes, apart from heavy metals test. Local requirements, solubility of film (Ph. Eur.). PDG sign off on 9 November 2010
Microcrystalline Cellulose (R1)	6	USP	X
Cellulose Powdered (R1)	6	USP	X
Citric Acid Anhydrous (R2)	6	Ph. Eur.	Harmonized by attributes, apart from heavy metals and bacterial endotoxins test. Local requirements are sterility (USP) and identification, water (Ph. Eur.). JP will not stipulate Al limit test. PDG sign off on 9 June 2010
Citric Acid Monohydrate (R2)	5B	Ph. Eur.	As for "Citric Acid Anhydrous"
Copovidone	4	JP	No commentary on harmonization in existing USP37/NF31 monograph
Croscarmellose Sodium	6	USP	X
Crospovidone	6	Ph. Eur.	Harmonized by attributes, apart from heavy metals and IR identification test. Identity test C (IR) and peroxides are not stipulated by USP. PDG sign off on 9 November 2010
Ethylcellulose (R1)	6	Ph. Eur.	Harmonized by attributes, apart from heavy metals. JP will not stipulate and has not taken part in harmonization. Local requirements are identification (Ph. Eur.). PDG sign off on 27 October 2009

Ethylparaben	6	Ph. Eur.	X
Gelatin (corr. 2)	6	Ph. Eur.	Harmonized by attributes, apart from the wording, "and/or enzymatic hydrolysis" in the definition. PDG sign off on 26 June 2014
Glucose Monohydrate (and Anhydrous)	6	Ph. Eur.	New monograph. Harmonized by attributes. Local requirements are first (specific optical rotation) and second identification (TLC, color reaction), pyrogens (Ph. Eur.), identification (color reaction), specific optical rotation (JP) and identification (IR) USP). PDG sign off on 4 November 2015
Glycerin	3	USP	No commentary on harmonization in existing USP37/NF31 monograph.
Glyceryl Monostearate	2	USP	No commentary on harmonization in existing USP37/NF31 monograph
Hydroxyethyl Cellulose	4/2	Ph. Eur.	No commentary on harmonization in existing USP37/NF31 monograph
Hydroxypropyl Cellulose	6	USP	Harmonized by attributes, except for description, description/solubility, viscosity, and heavy metals. Local requirements are for cloud point (Ph. Eur.) and lead (USP). PDG sign off on 27 June 2013
Hydroxypropyl Cellulose Low Substituted	4	USP	No commentary on harmonization in existing USP37/NF31 monograph
Hydroxypropylmethyl Cellulose (Hypromellose) (R1, corr. 2)	6	JP	Harmonized by attributes, local requirements are description (JP), appearance of solution (Ph. Eur.), and heavy metals (USP). PDG sign off on 1 July 2015
Hypromellose Phthalate	6	USP	X
Isomalt	6		Harmonized by attributes, except for heavy metals and optical rotation. Local attributes are identification (TLC, color reaction) (Ph. Eur.), identification (color reaction) (JP) and identification (TLC) (USP). PDG sign off on 27 June 2013

(*Continued*)

Table 9.4 (Continued)

Excipient monographs (revisions are designated in brackets)	Harmonization stage[a]	Coordinating pharmacopoeia	Comments[b]
Lactose Anhydrous (R4)	6	USP	Harmonized by attributes, except for heavy metals. Tests that are not stipulated are α,β anomers (USP), loss on drying (Ph. Eur.), tests that are non-mandated are α,β anomers (Ph. Eur.). Local requirements are identification B and C (USP), microbial contamination TYMC (USP), microbial contamination TYMC-Salmonella (JP), particle size distribution (USP), bulk and tapped densities (Ph. Eur.), and α,β anomers (USP will require a limit on the label). PDG sign off on 1 November 2010
Lactose Monohydrate (R2)	6	USP	Harmonized by attributes, apart from microbials and heavy metals test. Local requirements are identification test B and C (USP), LOD (USP), and particle size distribution (USP). Note the JP definition also covers granulated lactose (NMT 4.0% water) and USP definition also indicates that it may contain varying amounts of amorphous lactose. PDG sign off on 6 June 2008
Magnesium Stearate (corr. 3)	6	USP	Harmonized by attributes apart from microbials and SSA test. The JP will not stipulate limits for cadmium, nickel, or lead. PDG sign off on 27 June 2013
Mannitol	6	Ph. Eur.	Harmonized by attributes, except for heavy metals test. Local requirements are for second identification (Ph. Eur.), absence of *Salmonella* (Ph. Eur.), and functionality related characteristics. JP does not stipulate microbial contamination or bacterial endotoxins. PDG sign off on 6 June 2012[d]
Methylcellulose (R2 corr. 2)	6	JP	Harmonized by attributes. Local requirements are appearance of solution (Ph. Eur.), description (JP), and heavy metals (SP). PDG sign off on 1 July 2015

Methylparaben	6	Ph. Eur.	X
Petrolatum	4	USP	No commentary on harmonization in existing USP37/NF31 monograph
Petrolatum, White	4	USP	No commentary on harmonization in existing USP37/NF31 monograph
Polyethylene Glycol	4	USP	No commentary on harmonization in existing USP37/NF31 monograph
Polysorbate 80	6	Ph. Eur.	Harmonized by attributes. Non-harmonized attributes are identification by IR (EP) and heavy metals test (USP). PDG sign off on 14 November 2014
Povidone (R1 corr. 2)	6	JP	Harmonized by attributes. Local attributes are heavy metals (JP). Identification C and D and presence/absence of 1-vinylpyrollidin-2-one (Ph. Eur.) and identification B, C, D, and E and lead (USP). PDG sign off on 1 July 2015
Propylene Glycol	4	Ph. Eur.	No commentary on harmonization in existing USP37/NF31 monograph
Propylparaben	6	Ph. Eur.	X
Saccharin (R1)	6	USP	Harmonized by attributes, apart from description, melting range, limits on toluene sulfonamides, and heavy metals. Local requirements are identification tests A, B, D, and E (Ph. Eur.). Ph. Eur. will not stipulate limits for benzoate and salicylate. PDG sign off on 27 June 2013
Saccharin Calcium	6	USP	X
Saccharin Sodium (R1)	6	USP	X
Silicon Dioxide	4 (R)	JP	No commentary on harmonization in existing USP37/NF31 monograph
Silicon Dioxide, Colloidal	4 (R)	JP	No commentary on harmonization in existing USP37/NF31 monograph
Sodium Chloride (R3)	6	Ph. Eur.	Harmonized by attributes, apart from description, appearance of solution, arsenic, heavy metals, sterility, and bacterial endotoxins. PDG sign off on 6 November 2013

(*Continued*)

Table 9.4 (Continued)

Excipient monographs (revisions are designated in brackets)	Harmonization stage[a]	Coordinating pharmacopoeia	Comments[b]
Sodium Lauryl Sulfate	6	USP	New monograph. Harmonized by attributes. Local requirements are water, total alcohols (JP), and total alcohols (USP). PDG sign off on 14 November 2014
Sodium Starch Glycolate (R3)	5B	USP	Harmonized by attributes, except for identification (IR) and heavy metals. Local requirements are for appearance of solution (Ph. Eur.), identification – complies with pH test (Ph. Eur.), identification—formation of a suspension (Ph. Eur.) and identification—sodium flame test (USP). PDG sign off on 27 June 2013
Starch, Corn (R3)	6	USP	Harmonized by attributes. Local requirements are foreign matter (Ph. Eur./JP) and absence of *Salmonella* (Ph. Eur.) and absence of *Staph aureus*/*Pseudomonas aeruginosa* (USP). JP will not stipulate microbial limits. PDG sign off on 6 June 2012
Starch, Potato (R2)	6	Ph. Eur.	Harmonized by attributes. Local requirements are foreign matter (Ph. Eur.) and absence of *Salmonella* (Ph. Eur.). JP will not stipulate microbial contamination. PDG sign off not dated
Starch, Pregelatinized	3	JP	No commentary on harmonization in existing USP37/NF31 monograph
Starch, Rice (R1)	6	Ph. Eur.	Harmonized by attributes, except for foreign matter. Local requirements are for absence of Salmonella (Ph. Eur.). PDG sign off on 6 November 2013
Starch, Wheat (R2)	6	Ph. Eur.	Harmonized by attributes. Local requirements are foreign matter (Ph. Eur.) and absence of *Salmonella* (Ph. Eur.). JP will not stipulate microbial contamination. PDG sign off date not recorded
Stearic Acid	6	Ph. Eur.	Harmonized by attributes apart from nickel limit test. PDG sign off on 3 June 2008

Sucrose (R1)	6	Ph. Eur.	Harmonized by attributes apart from identification and color value test. PDG sign off on 27 June 2013
Sterile Water for Injection in Containers	3	USP	No commentary on harmonization in existing USP37/NF31 monograph
Talc	6	Ph. Eur.	X
Titanium Dioxide	5A2	JP	No commentary on harmonization in existing USP37/NF31 monograph

Accurate at the time of writing, that is, 2Q2016.
X, status not clear.
[a] See Figure 9.1.
[b] USP generally comments on labeling, packaging, and storage and USP reference standards.
[c] USP general chapter <1191> indicates pre-stage 5 (regional harmonization) status but specific USP monograph highlights significant elements of harmonization.
[d] Date in PDG sign off document is not clear.

In conclusion, pharmacopoeial harmonization, which is an admirable goal in itself, has not really delivered the anticipated global benefits. Indeed, the whole area of pharmacopoeial harmonization remains a complex one with little reason to believe that progress will be substantive in the near future. Indeed, the requirements by some BRIC nations, for example, China, Russia, India, Brazil, and other regional countries, for example, Argentina, for compliance with their national pharmacopoeias, have made the goal of globally harmonized submissions even less likely.

References

1 ICH Q6A. 1999. Specifications: Test procedures and acceptance criteria for new drug substances and new drug products: Chemical substances. Current step 4 version, October 6, 1999.
2 ICH Q6B. 1999. Specifications: Test procedures and acceptance criteria for biotechnological/biological products. Current step 4 version, March 10, 1999.
3 Moore K. 2012. Overview of the Pharmacopoeial Discussion Group: Harmonization from a Compendial Perspective. http://www.irvinepharma.com/pdf/Kevin_Moore_PDG_Presentation.pdf. Accessed on September 23, 2014.
4 ICH Q4B. 2007. Evaluation and recommendation of pharmacopoeial texts for use in the ICH regions. Current step 4 version, November 1, 2007.
5 ICH Q3D. 2014. Guideline for elemental impurities. Current step 4 version, December 16, 2014.
6 Schoneker D. 2015. ICH Q3D: Elemental impurities: How to prepare your company to comply with regulatory expectations, ExcipientFest Asia, March 25–26, 2015, Renaissance Shanghai Zhongshan Park Hotel, China.
7 Das TK. 2012. Protein particulate detection issues in biotherapeutics development: Current Status. *AAPS PharmSciTech*, 13, 732–746.
8 Quershi SA, McGilveray IJ. 1995. A critical assessment of the USP dissolution apparatus suitability test criteria. *Drug Development and Industrial Pharmacy*, 21, 905–992.
9 Quershi SA, McGilveray IJ. 1999. Typical variability in drug dissolution testing: Study with USP and FDA calibrator tablets and a marketed drug (glibenclamide) product. *European Journal of Pharmaceutical Sciences*, 7, 249–258.
10 Armenante P, Muzzio F. 2005. Inherent Method Variability in Dissolution Testing: The Effect of Hydrodynamics in the USP II Apparatus A Technical Report Submitted to the Food and Drug Administration. http://www.fda.gov/ohrms/dockets/ac/05/briefing/2005-4187B1_01_04-Effect-Hydrodynamics.pdf. Accessed on May 16, 2016.

11 Collins CC, Nair RR. 1998. Comparative evaluation of mixing dynamics in USP apparatus 2 using standard USP vessels and peak vessels. *Dissolution Technology*, 5, 7–18.

12 Quershi SA. 2006. Comparative impact of stirring and shearing in drug dissolution testing with USP paddles and crescent shaped paddles. *Dissolution Technology*, 13, 25–30.

13 Quershi SA, Shabnam J. 2001. Cause of high variability in drug dissolution testing and its impact on setting tolerances. *European Journal of Pharmaceutical Sciences*, 12, 271–276.

14 Zhang Y, Bredael G, Aremante PM. 2013. Dissolution of prednisone tablets in the presence of an arch-shaped fiber optic probe in a USP dissolution testing apparatus 2. *Journal of Pharmaceutical Sciences*, 102, 2718–2729.

15 Wang Y, Armenante PM. 2012. A novel off-center paddle impellor dissolution testing system for reproducible dissolution testing of solid dosage forms. *Journal of Pharmaceutical Sciences*, 101, 746–760.

16 Holte Ø. 2011. The European Pharmacopoeia Draft on Large Sample Sizes, PQRI Workshop on Sample Sizes for Decision Making in New Manufacturing Paradigms, September 12–13, 2011. http://pqri.org/conferences-and-workshops/. Accessed on July 21, 2017.

17 Leveau P. 2012. The European Pharmacopoeia: Harmonisation and Innovation, 44ème Congrès International SFSTP, June 6–7, 2012, Montpellier, France.

18 Huang C-Y, Ku MS. 2010. Asymmetry effect of particle size distribution on content uniformity and over-potency risk in low-dose solid drugs. *Journal of Pharmaceutical Sciences*, 99, 4351–4362.

19 Lunney PD, Anderson CA. 2009. Investigation of the statistical power of the content uniformity tests using simulation studies. *Journal of Pharmaceutical Innovation*, 4, 24–35.

20 Chowhan ZT, Amaro AA, Ong JTH. 1992. Punch geometry and formulation considerations in reducing tablet friability and their effect on *in vitro* dissolution. *Journal of Pharmaceutical Sciences*, 81, 290–294.

21 FDA. 2012. Guidance for Industry Pyrogen and Endotoxins Testing: Questions and Answers. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER) Center for Veterinary Medicine (CVM) Center for Devices and Radiological Health (CDRH) Office of Regulatory Affairs (ORA), June 2012. http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm. Accessed on September 14, 2014.

22 Long AG. 2013. Pharmacopoeial harmonization update. USP Convention, June 27, 2013.

23 USP. 2013. Monograph Modernization, December 13, 2013. http://www.usp.org/usp-nf/key-issues/monograph-modernization. Accessed on May 16, 2016.

24 EMA. 2010. Water for Injection (EDQM), April 1, 2010. http://www.gmp-publishing.com/en/gmp-news/gmp-aktuell/water-for-injection-edqm/page/33.html?layout=print. Accessed on September 23, 2014.
25 Benoit-Guyod F. 2012. EU GMP Requirements and Inspections of API Manufacturers Organized by EDQM. IPA/EDQM/WHO Mumbai Conference, September 28, 2012. http://www.ipapharma.org/events/edqmphotospresentation2012/12.pdf. Accessed on September 23, 2014.
26 ICH. 2009. Final Business Plan. Q3D: Impurities: Guideline for Elemental Impurities, Dated July 17, 2009. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3D/Q3D_Final_Business_Plan.pdf. Accessed on September 14, 2014.
27 Shah V. 2013. Progressive applications of dissolution: Its impact, and implications in the pharmaceutical world. *Journal of Pharmaceutical Sciences*, 102, 2895–2897.

10

ICH Q5A

Viral Safety of Biotechnology Products

Daniel Galbraith

BioOursource Ltd., Glasgow, UK

10.1 Introduction

Biotechnology products (biologicals) are produced using mammalian, insect, or microbial cell lines and are typically unsuitable for harsh terminal sterilization approaches. This has emphasized the need for assuring safety from adventitious agent contamination throughout the manufacturing process. Many of the techniques of manufacture of biotechnology products were first implemented for producing vaccines, and the knowledge gained in production of vaccines has also been important in implementing approaches for ensuring the safety and quality of biotechnology products in general. Viral vaccines in the vast majority of cases have been safe, although there have been a small number of cases of contaminated products or processes. These occurrences have led to the creation of a number of guidelines for vaccines that have addressed the reduction of such safety risks. When the ICH created the guidelines for the safety of biotechnology products, the experience obtained from the vaccine guidelines was taken into consideration. In the resulting ICH Q5A guideline (viral safety evaluation of biotechnology products derived from cell lines of human or animal origin) [1], the risks of contamination of biotechnology manufacturing processes are explained, and the approaches that are expected to be taken in mitigating these risks are described.

Biologicals are fundamentally different from small molecule drugs as the former are produced in living cells, often modified using recombinant DNA technology. For many years, viral vaccines have been manufactured by growing the viruses in cells. Many of these products have a terminal step in the process that is capable of inactivating any remaining infectious agents, including the virus itself and any contaminating microbes. This effectively makes vaccines a

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low safety risk and a product that can be used even in young healthy children. Most biologicals, on the other hand, are unable to withstand the traditional terminal sterilization steps employed by the vaccine industry, and therefore other means by which to ensure the product is free of infectious agents have had to be developed.

10.2 Overall Approach for Assuring Viral Safety

As described in Q5A(R1), the industry has evolved the safety triangle (Figure 10.1) that comprises the three approaches that are deemed to be required to ensure safety of biologicals.

Raw materials are defined in Q5A(R1) as the production cell line and other raw materials, including media components, and "in-process testing" includes testing for adventitious agents at appropriate steps of production (i.e., lot release). The limitations of testing as a means of assuring safety are acknowledged, and such deficiencies are mitigated through implementation of process steps for removing potential viral contaminants. The third part of the triangle therefore includes validation of purification (clearance) steps for removing and/or inactivating viruses.

The three approaches displayed in Figure 10.1 are linked through risk assessment of both the product and the process. All biologicals are required to have a viral risk assessment (e.g., European Pharmacopoeia 5.1.7 [2]; see also Chapter 14) prepared by the drug developer at a very early stage. This is scrutinized by the regulatory authorities as part of the evaluation of an application for clinical trials. The specific language in Q5A(R1) is "The approach used by manufacturers in their overall strategy for ensuring viral safety should be explained and justified. In addition to the detailed data which is provided, an

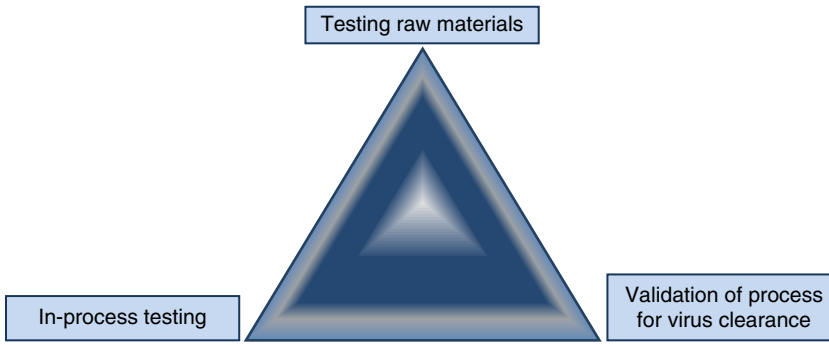


Figure 10.1 Safety testing triangle for biologicals. *Source:* Reproduced with permission of Professor David Onions.

overall summary of the viral safety assessment would be useful in facilitating the review by regulatory authorities" [1].

As described in Q5A(R1), potential viral contaminants may be introduced either through the production cell or the production process. Cell lines such as Chinese hamster ovary (CHO) and the mouse cell lines NS0 and Sp2/0 are, by far, the most commonly used in the production of complex biologicals including monoclonal antibodies. Regulatory authorities have, over a number of years and by reviewing many different product applications, become aware of the risks associated with products derived from such cell substrates. Because of this, the testing requirements for products derived from these *well-characterized* cells are almost systematized.

The risks associated with the manufacture of biologicals with CHO cells, for example, are not theoretical. There have been a number of reported cases where virus contamination has been detected during the production of biologicals, and these cases have led to modifications in the expectations for testing conducted at the unprocessed bulk stage. For instance, the parvovirus mouse minute virus (MMV) was reported [3] to be a contaminant in batches of product in the 1990s, and subsequently tests for this virus have been expected. Recent occurrences of contamination by this virus have been reported [4, 5], confirming the need for such testing. More recently in 2013, Genzyme reported [6] a contamination of a CHO production process with the calicivirus, vesivirus. This is actually the second report of a contamination of a CHO cell process with this virus, as the first report of such a contamination appeared two decades earlier [7]. A viral safety testing panel for CHO cell products should therefore include an assay capable of detecting this virus. These are not the only types of viral contaminations observed in CHO cell manufacturing processes [8]. Awareness of potential risks of virus contaminations applicable to various production cell types is continually changing, and the industry must keep up to date with the current thoughts on the most appropriate testing panels.

As mentioned previously, raw material testing (Figure 10.1) includes testing of the production cell line (master cell bank (MCB), working cell banks (WCBs), and end of production cells/cells at the limit of *in vitro* age) and the growth media and its components used in the culture of the cells. In considering raw materials, special emphasis is given to any animal-derived materials, as these are believed to be the most likely source for introduction of adventitious contaminants.

10.3 Cell Line Qualification

ICH Q5A(R1) describes the use of cell banks for production, where a few hundred vials of cells are prepared as an MCB and a single vial from the MCB is expanded to create one or more WCB. It is acknowledged in Q5A(R1) that the

Table 10.1 Tests performed on cell banks.

Detection end point	MCB	WCB	CAL[a]
Retrovirus and endogenous virus			
Infectivity	+	–	+
Electron microscopy	+	–	+
Reverse transcriptase	+	–	+
Other virus-specific tests	+	–	+
Adventitious virus tests			
In vitro assay	+	+	+
In vivo assay	+	–	+
Antibody production tests	+	–	–
Other virus-specific test	+	–	–

Source: ICH Q5A(R1) [1]. Courtesy of International Conference on Harmonisation Q5A(R1).
[a] Cells at the limit of *in vitro* age (CAL) are cells harvested at the end of the upstream production process, or a few passages beyond.

production cell line is a potential source of viral contaminants. The production cell banks are therefore expected to be tested thoroughly for possible viral contaminants. Viruses are a complex mix of different types and as such are impossible to detect by means of a single assay methodology. Consequently, a panel of orthogonal virus detection assays are used, detailed in the testing of cell banks in the Q5A(R1) guideline, and summarized in Table 10.1 (see the original guidance document for additional details concerning this testing).

10.3.1 Detection of Endogenous Retroviruses

Endogenous retroviruses are of concern as these viruses are present in the rodent production cell lines commonly employed for biologicals production, including CHO cells. As retroviruses can replicate at low levels or are sometimes completely dormant (existing as a few thousand nucleotides in the host cell genome), it is expected that a variety of orthogonal testing methods be employed. Infectivity testing is the most useful method to identify retroviruses of concern. Since there are minimal or no visible cell morphology changes following retrovirus infection of the human detector cell lines commonly used in virus screening assays (MRC-5, HEK-293, and HeLa), infectivity assays unique to retroviruses have been developed. For instance, focus formation assays allow detection of retroviruses through changes to the cellular morphology (formation of transformed foci) resulting from retrovirus activation of a defective sarcoma virus (S^+L^-). In this way both identification and quantitation of virus

can be achieved. For S^+L^- assays, typically mink (ATCC CCL-64.1 MiCl1 S+L–) or cat (ATCC CRL-2032 PG-4 S+L–) cell lines are used. These assays specifically detect xenotropic or amphotropic murine retroviruses (i.e., retroviruses that grow in species other than the host cell of origin), as these are the retroviruses of most concern from a patient safety viewpoint. The cat S^+L^- cell line is generally thought to be more sensitive at detection than the mink S^+L^- cell line [9]. These S^+L^- cells harbor a replication-defective murine sarcoma virus, and upon infection with a second retrovirus from the test material, the defective virus is "rescued" and causes the detector cells to undergo rapid replication, forming small foci that are visible under light microscopy. Each focus is equivalent to a single infectious virus.

Other retrovirus detection assays that are employed include cocultivation assays using *Mus dunni* [10] or nonhuman or human cells and the XC plaque formation assay. These assays address a special retroviral risk associated with the use of production cells of murine origin, such as the NS0 or Sp2/0. Mouse cell lines have a complex array of endogenous retroviruses that are expressed and these assays are able to identify the different types. This is important as some murine retroviruses can be considered low risk as they can only infect rodent cells (ecotropic), whereas others can infect any cell type (amphotropic). The *Mus dunni* cells are able [10] to support the replication of all four classes of murine leukemia virus (MuLV) (i.e., ecotropic, xenotropic, amphotropic, and mink cell focus-forming (MCF)). To increase sensitivity of detection of virus produced from the cells of an MCB, the cells are first cocultivated for a period of up to 4 weeks with the *Mus dunni* cells. During this period when the two cell types are in close contact, any infectious retrovirus shed by the MCB cells can infect and amplify in the susceptible *Mus dunni* cells. A harvest of the supernatant from the mixed culture can then be used in a subsequent end point such as an S^+L^- assay to detect the propagating retrovirus. It is thought that this process can increase the sensitivity of detection of these methods by 10-fold over an S^+L^- assay without the initial cocultivation. Alternatively, specific monoclonal antibodies are available to identify MuLV types on the *Mus dunni* cell surface.

The XC plaque assay is a cell detection system to detect ecotropic MuLV; in this case, the detector cells (XC) are rat in origin. The test sample is first inoculated onto the mouse embryo cell line SC-1 to amplify any ecotropic retrovirus present. The inoculated SC-1 culture is then UV irradiated and a monolayer of XC cells is overlaid onto the irradiated SC-1 cells. If ecotropic virus is present, giant multinucleated XC cells (syncytia) are observed in the cultures. These are enumerated and represent an indication of the titer of retrovirus present at the time of UV irradiation.

The electron microscope has been used for many years in virology to provide fine detail of virus structure. With respect to the detection of retroviruses, the electron microscope is particularly useful. The method as applied to fixed cells

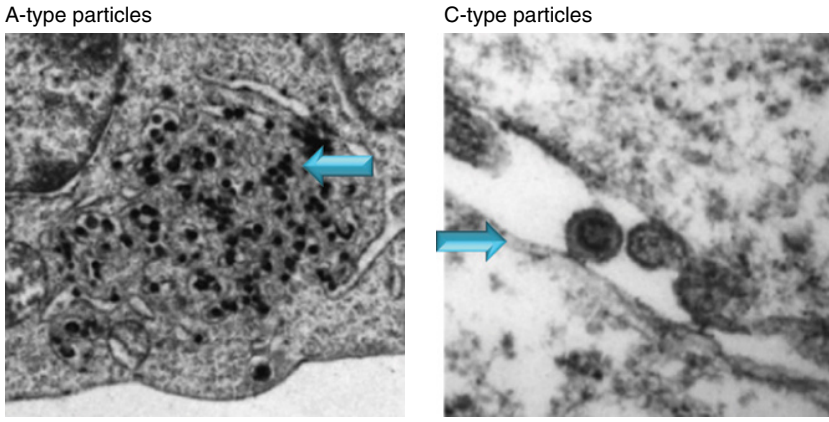


Figure 10.2 Electron micrographs of CHO cells demonstrating A- and C-type particles.

allows the observation of virus particles (80–100 nm) that are intracellular and also following release of the virus through budding from the cell membrane. CHO cells are known [11] to produce retrovirus particles classified as A-type (intracellular) and as C-type (enveloped following budding from the cell membrane) (Figure 10.2).

When testing the cells of the MCB using electron microscopy, one vial is thawed and used to produce growing cells that are then fixed. Ultrathin sections of the cells are taken using a microtome. The slices through the center of the cells are stained and prepared for examination. The cells at metaphase are selected using a transmission electron microscope (TEM). Normally 100–200 such cell profiles are inspected and the percentage of cells expressing A- and C-type particles is calculated. For CHO cells, A-type particles can be seen in up to 20% of cells in the bank, while C-type particles are observed with considerably less frequency.

The electron microscope can also identify other virus particles in the cells and as such can be a useful tool if there is an unexpected latent virus present. Such latent viruses may include members of the *polyomaviridae* and *herpesviridae* families. Although an extremely useful tool, the electron microscope does have a significant drawback in the analysis of samples; the volume able to be tested is incredibly small. For example, evaluation of 200 cell profiles represents a tiny sample relative to the 10^6–10^7 cells normally present in a cell bank vial. This represents only 0.0002% of the population of cells in a vial, and there is a good chance of missing contamination using the method, unless a high percentage of the cells are infected (which is unlikely in latent infection).

Biochemical assays and polymerase chain reaction (PCR)-based assays for the enzyme reverse transcriptase (RT) are also useful as retrovirus detection end points [12]. This enzyme is used during the retrovirus life cycle to translate

the genomic RNA of the virus to a DNA copy that can integrate into the host cell DNA and is a relatively specific marker for retroviruses. In addition, as the number of viruses and viral-derived products increases, so does the amount of RT enzyme present. The method of choice is the polymerase-enhanced RT (PERT) assay, in which a synthetic template RNA, in the presence of viral RT enzyme, is used to synthesize a DNA copy that can be measured by PCR [9]. The PERT assay is sensitive, having the ability to detect less than 1000 virus particles in a sample. One downside of the assay is the chance of obtaining false positive results due to the ability of certain mammalian nuclear enzymes to mimic RT activity, if present at high levels. Care should be taken to ensure that correct controls are included in the assays to account for this possibility.

10.3.2 Detection of Adventitious Viral Contaminants

PCR-based end point assays are also employed for the detection of a variety of adventitious viruses of concern for which cell-based infectivity assays are not available. Included in the list of viruses detected by PCR testing are the retroviruses HIV and HTLV, hepatitis B and C viruses, herpesviruses including Epstein–Barr virus (*human herpesvirus 4*), and human polyomavirus (e.g., JC and BK virus). Use of PCR assays for testing of cell banks is discussed further in the succeeding text.

The tests for non-endogenous or adventitious virus also include a variety of cell-based infectivity screening methods that are designed to detect an array of viruses that may have infected the cell banks. These include the *in vitro* virus screen, the *in vivo* virus screen, and the antibody production assays.

10.3.3 *In Vitro* Screening Assay for Adventitious Virus

The so-called *in vitro* assay is widely used to detect viruses that are able to (i) infect and propagate in detector cell cultures and (ii) cause a discernible effect in one of the three end points available (i.e., visualization of cytopathic effect, hemadsorption or hemagglutination of red blood cells). A sample of cell lysate from the banked cells is inoculated onto growing detector cell monolayers. The detector cells used, as a minimum, should include human diploid cells, monkey kidney cells, and a cell line of the same species as the production cells. Other types of detector cell lines that may be considered are listed in Table 10.2.

The optimal duration (i.e., 14 or 28 days) of the *in vitro* assay depends on a number of factors. The duration is not specified in Q5A(R1). If the production cell line is of non-primate origin and is not known to support the growth of human cytomegalovirus (CMV), the observation period for the inoculated cultures can be limited to 14 days according to guidance documents such as the 1993 US FDA Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals [13]. If the cells are suspected to support the growth of human CMV, the recommended observation period is 28 days. Although many

Table 10.2 Examples of detector cell lines used in *in vitro* virus screening assays.

Cell line	Species of origin	Notes
324K	Human (*Homo sapiens*)	The cells are particularly susceptible to mouse minute virus (MMV) and may be added to assays for testing CHO production cells and unprocessed bulk harvests
A549 CCL-185	Human (*Homo sapiens*)	Robust cellular growth makes this an ideal detector line. Susceptible to both adenoviruses and herpesviruses but not extensively documented as being able to detect other virus types
BHK-21 CCL-10	Syrian golden hamster (*Mesocricetus auratus*)	Commonly used to detect arboviruses (insect-borne viruses) and some vaccinia viruses and herpesviruses
CHO-K1 CCL-61	Chinese hamster (*Cricetulus griseus*)	Used in testing CHO production cells, detects reoviruses and vesivirus
HEK-293 CRL-1573	Human (*Homo sapiens*)	Unusual mixed morphology of the cells can be observed. Able to support the growth of a number of virus types but particularly adenoviruses
HeLa CCL-2	Human (*Homo sapiens*)	Used for detecting adenovirus and a variety of other viruses
LLC-PK1 CL-101	Pig (*Sus scrofa*)	Often used for porcine parvovirus detection or for testing production cells that have been exposed to porcine material such as trypsin
MDBK CCL-22	Cow (*Bos taurus*)	Widely used to detect bovine viruses such as bovine viral diarrhea virus. Useful in the testing of bovine sera for viral contamination
MDCK CCL-34	Dog (*Canis familiaris*)	Used to grow influenza virus for vaccine purposes by some manufacturers and useful for detecting members of the *Orthomyxoviridae* family
MRC-5 CCL-171	Human (*Homo sapiens*)	Diploid cell used for detecting measles, cytomegalovirus, and a variety of other viruses [13]
RK13 CCL-37	Rabbit (*Oryctolagus cuniculus*)	Used exclusively for the detection of herpes B virus *(Macacine herpesvirus 1*), a possible contaminant of primary monkey cells. The cells also detect other virus types
Vero CCL-81	African green monkey (*Cercopithecus aethiops*)	Detects a variety of viruses infectious for humans and primates [13]

viral contaminants can be observed in the *in vitro* assay before day 5 post-inoculation, certain viruses such as CMV and measles virus can be slower to exhibit CPE, and therefore for cell line characterization, it is typical to use the longer (i.e., 28-day) observation period to provide the greatest chance for detecting a low level contaminant.

The inoculated cultures are maintained for 14 or 28 days to allow the virus time to propagate. During this period, cytopathic effects may be observed using light microscopy. At various time points in the assay, samples may also be tested for hemadsorption and hemagglutination.

Hemadsorption is the ability of erythrocytes to firmly bind to cell surface glycoproteins expressed during infection of the cells with certain types of viruses. Each virus family or species has slightly different glycoproteins that, in turn, can have different affinities for erythrocytes from different animal species. These glycoproteins also have the ability to preferentially bind erythrocytes at different temperatures (Table 10.3). Typically a low range (2–8°C) and a high range (ambient or 37°C) are evaluated. Free viruses shed into culture media can also bind to erythrocytes and cause an effect known as hemagglutination. In this case, rafts of erythrocytes become held together by the virus glycoproteins. These rafts may remain suspended in solutions or may sink to the bottom of a 96-well test plate and form an uneven-shaped carpet (button) of erythrocytes easily identified visually (samples from uninfected samples form a tight button of erythrocytes). It should be noted that there must be sufficiently high virus population present to allow this; therefore this is likely the least sensitive of the end points used in the *in vitro* screen. Many virus families can cause the formation of hemagglutination, and this is recognized as a key identification feature of some viruses (e.g., orthomyxoviruses).

It should be noted that many of the viruses of concern to biologicals production (e.g., adenoviruses, bunyaviruses (e.g., Cache Valley virus), caliciviruses (e.g., vesivirus 2117), circoviruses (e.g., porcine circoviruses 1 and 2), hepadenoviruses (i.e., hepatitis B), picornaviruses (e.g., rhinoviruses, enteroviruses), and rhabdoviruses (e.g., rabies)) do not cause positive end point responses in hemadsorption and hemagglutination assays and are therefore not detectable using these end points. As the typical screening assay uses a fixed panel of erythrocytes (such as human or monkey, guinea pig, and chick), in a practical sense, flaviviruses (e.g., bovine viral diarrhea virus, West Nile virus) and herpesviruses are also not typically detectable using these end points.

Hemagglutination and hemadsorption are valuable confirmatory end points for the detection of the specific virus families mentioned in Table 10.3; however care should be taken to ensure that the erythrocyte preparation used in the assays contains cells that are capable of agglutinating in the presence of a positive control virus. The erythrocytes, particularly if not fresh or have been held at high temperatures, may lose their ability to function in the assays.

Table 10.3 Hemagglutinin characteristics of some virus types.

Virus (family)	Hemagglutination/ hemadsorption	Comments
Influenza A, B, C (*Orthomyxoviridae*)	+/+	Human, guinea pig, turkey, or chick erythrocytes give positive results at both low and high temperature ranges; however this can vary between viral isolates
Parainfluenza 1–3 (*Paramyxoviridae*)	+/+	Human, guinea pig, and chick erythrocytes give positive responses, with the guinea pig being the preferred source. Agglutination occurs at both temperature ranges
Mumps virus (*Paramyxoviridae*)	+/+	Human erythrocytes can work but avian, particularly goose, erythrocytes provide the best results. Agglutination is stronger at the lower temperature range
Rubella virus (*Togaviridae*)	+/+	Human, goose, and chick erythrocytes give positive results
Sindbis virus (*Togaviridae*)	+/–	Agglutinates goose and sheep erythrocytes
Measles virus (*Paramyxoviridae*)	+/+	Monkey (rhesus or *Cercopithecus*) erythrocytes give positive results Agglutination is enhanced at higher temperatures (37°C)
West Nile virus (*Flaviviridae*)	+/–	Agglutinates goose and sheep erythrocytes
Herpes/CMV (*Herpesviridae*)	+/–	No obvious hemadsorption but will agglutinate sheep erythrocytes at high virus titer
Mouse minute virus (*Parvoviridae*)	+/–	Guinea pig and mouse erythrocytes give positive agglutination results at both temperature ranges
Reo type 2 (*Reoviridae*)	+/–	Weak and variable agglutination with monkey, guinea pig, and chick erythrocytes at both temperature ranges

As the *in vitro* assay is a screening assay, a positive outcome for a test sample must be followed up using orthogonal methods to enable identification of the contaminant. Historically, this has been accomplished using TEM and specific antisera directed against virus families or species. Confirmation of proposed identity has involved specific PCR assays. More recently, these historical approaches have been superseded by next-generation (deep) sequencing.

Recent publications from industry experts have suggested a number of changes to the *in vitro* assay, including expanding the panel of cell lines used to potentially increase assay sensitivity for certain viruses. In particular, this has been recommended in the case that the *in vivo* methods (*in vivo* screen, antibody production) are reduced or eliminated. In addition, consideration might be given to the incubation of indicator cells at lower temperatures (e.g., 33–35°C) to enhance detection of respiratory viruses that might propagate better in the lower temperatures of the upper respiratory tract (e.g., rhinoviruses). Furthermore, consideration might be given to optimizing the test for detection of cell-associated viruses. Cell debris may interfere with the test but may contain cell-associated viruses. It might be appropriate to include additional end points, for example, immunofluorescent staining for specific viruses of greatest concern or broad PCR mass spectrometry for general detection following amplification in culture. The modifications to the standard *in vitro* assay suggested previously would not require revision of Q5A(R1), as there currently is not much detail to be found in this guidance regarding the *in vitro* virus screening method. The biologics manufacturer is relatively free to optimize the method in order to provide the best sensitivity for the specific viruses of concern, taking into account the production cell and any animal-derived raw materials that might be used in the upstream process.

10.3.4 Detection of Bovine and Porcine (i.e., Species-Specific) Viruses

It is likely that the cells commonly used in biologicals production (CHO, NS0, Sp2/0, HEK-293) were at one time in their histories cultivated using animal-derived materials such as bovine serum or porcine trypsin. Naturally, as these materials are derived from an animal source, there is the risk that animal viruses may be transmitted to the production cells during cultivation. Testing for such viruses is not described in Q5A(R1), although the requirement perhaps is included in Table 10.1 of that guidance under the category of "other virus-specific tests." Notwithstanding the silence of Q5A(R1) with regard to the inclusion of specific tests for cell banks exposed at some point to animal-derived materials, such testing is expected to be performed.

Guidelines have been in place for many years to cover the testing of potential viral contaminants in animal-derived raw materials, but the most comprehensive and the one typically followed is that presented in the US Code of Federal Regulations (CFR) 9 CFR 113 parts 46 [14] and 47 [15]. These outline the viruses that should be tested for in batches of bovine serum, porcine trypsin, and other raw materials of animal origin and proscribes the test methodology that should be used. The test uses at least two cell lines: Vero (*Cercopithecus* monkey kidney) and a cell line derived from the species of origin of the test material. After the sample is inoculated onto the detector cells, the assay lasts 3 weeks with a proscribed number of passages of the cultures. The end points

are cytopathic effect, hemadsorption, and immunofluorescent staining, which probe for a number of viruses specified in the CFR. Although this method was specifically intended for testing raw materials, it has also been applied to testing of cell banks exposed to such animal-derived materials.

10.3.5 *In Vivo* Virus Screen for Adventitious Virus

The so-called *in vivo* methods represent a historic method of virus detection employing animals as the host organism for the detection of viruses. Per Q5A(R1), the laboratory animals to be inoculated include embryonated hens' eggs and adult and suckling mice. Although not specifically mentioned in Q5A(R1), guinea pigs are sometimes also inoculated. The tests are intended to detect viruses that are not detectable using the *in vitro* screening assay. These animals are theoretically susceptible to a variety of virus species, as illustrated in Table 10.4.

Adult mice are normally used at a weight of 15–20 g and a minimum of 10 animals are used in each study. Many different mouse strains are available but generally CD-1 or Balb/c is used. The mice are derived from a closed colony where the health status of the animals is monitored to minimize the risk of

Table 10.4 Susceptibility of various laboratory animals to viruses.

Animal	Comment
Adult mice	Sensitive to a large number of viruses but particularly noted for lymphocytic choriomeningitis virus, coxsackieviruses, flaviviruses, rhabdoviruses, and togaviruses.
Suckling mice	Mice are tested at less than 24 h old. Noted for their sensitivity to arboviruses, coxsackieviruses, human enteroviruses and other picornaviruses, alphaviruses, herpesviruses, flaviviruses, and rabies virus
Hens' eggs	Eggs are sourced from specified pathogen-free chicken flocks. Eggs are set and inoculated using different routes. Using the allantoic route with 10- to 11-day-old embryos, orthomyxoviruses (influenza viruses A and B), paramyxoviruses (mumps virus, measles virus, parainfluenza viruses 1–3), and alphaviruses may be detected
	Using the yolk sac route with 6–7-day-old embryos, herpesviruses (HSV), picornaviruses, poxviruses, and rhabdoviruses (rabies virus) may be detected
Guinea pigs	Young adult animals are commonly used for this test. Guinea pigs are not noted for their susceptibility to many virus families but are able to be infected by orthomyxoviruses, reoviruses, and filoviruses. It should be noted that filovirus would be an extremely rare and much unexpected risk of contamination

obtaining false positive results. The animals can be inoculated via a number of routes (i.e., intraperitoneal, intramuscular, subcutaneous, and intracranial). The route is not specified in Q5A(R1). Most commonly the intraperitoneal and intramuscular routes are evaluated. These routes are likely to be the most effective to introduce virus and are also the least severe and injurious for the animals. Following inoculation, the mice are monitored for 28 days in order to observe any signs of infection. Animals can be excluded from the study if they show signs of injection-related trauma. Mice can show signs of viral pathology within a matter of hours. This may involve changes in the state of their fur, the alertness of their eyes, and their general demeanor when compared with vehicle control animals. Animals displaying obvious signs of clinical illness are euthanized prior to any significant pain or suffering. The vital organs of these animals are retained frozen for further investigation per Q5A(R1). A variety of viruses are theoretically detectable using this route (Table 10.4).

Suckling mice are inoculated with the test materials into the cranial cavity, into the peritoneum, and into the hind muscle and then returned to their mothers to suckle. The animals are monitored for health over a 14-day period. The suckling animals can be more prone to injury during the inoculation procedure and animals with injection trauma can be removed from the study. A minimum of 20 suckling mice are used in each study. This portion of the *in vivo* assay is considered to be useful for detection of arboviruses and a variety of other viruses (Table 10.4).

Embryonated hen's eggs are obtained from specified pathogen-free flocks. Globally there are only a few suppliers of these eggs and their main customers are influenza vaccine manufacturers. The demand for these eggs therefore peaks in the months when the vaccine is being produced (Northern hemisphere: January–June) and is low in the other months. Eggs are shipped at a cooled temperature to prevent the embryos from developing en route. This can become an issue in the summer months if the eggs become overheated and this can lead to embryo death. On receipt the eggs are "set" by being placed into a humidified incubator where the development of embryos starts. The developing embryos are monitored by "candling" the eggs against a bright light to show the blood vessels and to monitor the maturing of the embryo. The egg at this stage consists of different regions: including the yolk sac, allantoic fluid, amniotic sac, chorioallantoic membrane (CAM), and the embryo itself. The allantoic fluid is the most commonly used and simplest route of administration. This route is optimal for the detection of paramyxoviruses, flaviviruses, and rhabdoviruses (Table 10.4). Many avian viruses can be isolated using this method. The other commonly used route is the yolk sac. Many mammalian viruses are isolated using this method (Table 10.4). The amniotic sac is used for primary isolation of influenza A virus and the mumps virus. Inoculation of the CAM is mainly employed for the growth of herpesviruses and poxvirus where lesions (pocks) are present as a gray white area in the transparent CAM. This

inoculation route would only be used where there is a high risk of contamination with pox or herpes virus.

There has been considerable debate concerning the usefulness of the *in vivo* assay over the past decades, and there have been calls for the possible elimination of this test in the light of efforts to reduce the use of live animals for research. There has been no instance in which a viral contaminant from a biologicals production process has been detected using the *in vivo* assay. Recent publications [16, 17] have provided empirical evidence that the *in vivo* assay provides relatively low sensitivity for viral detection compared to the *in vitro* screen. These publications describe detection of viruses spiked into test samples and do not reflect actual process contamination events. As mentioned previously, the intent of the *in vivo* assay was to detect viruses that were not detectable using the *in vitro* screen. In the Gombold et al. study [17], only influenza virus and vesicular stomatitis virus were detected at lower amounts by the *in vivo* screen than the *in vitro* screen. In addition, the adventitious agents of greatest concern from a biologicals safety point of view would be those that will propagate and achieve a high titer during the production growth cycle. By definition, these viruses should grow well in culture and should be easily detected in the *in vitro* assay, provided that a detector cell of the same species as the production cell is employed. The regulatory authorities are the final arbiters of the types of testing methodologies that should be applied to the testing of biologicals. Until such time as the Q5A is revised, the *in vivo* test will remain on the list of required testing for cell banks.

10.3.6 Antibody Production Tests

Rodent cell lines, and the Chinese hamster cell in particular, are the most commonly used cells in producing biologicals. Early on in the history of biologicals, a committee arrived at a list of potential viruses of concern that included 16 "mouse" viruses (ectromelia virus, mouse rotavirus, murine encephalomyelitis virus, Hantaan virus, lymphocytic choriomeningitis virus (LCMV), lactate dehydrogenase elevating virus, minute virus of mice, mouse adenovirus, mouse hepatitis, mouse salivary gland virus (murine cytomegalovirus), murine pneumonia virus, mouse polyomavirus and K virus, reovirus 3, Sendai virus, and mouse thymic virus), five "hamster" viruses (LCMV, reovirus 3, Sendai virus, murine pneumonia virus, and simian virus 5), and nine "rat" viruses (Hantaan virus, murine encephalomyelitis virus, Kilham rat virus, murine pneumonia virus, reovirus 3, Sendai virus, Toolan virus, rat coronavirus, and sialodacryoadenitis virus). The assay developed to detect these viruses was called the antibody production assay (e.g., the mouse antibody production (MAP) assay, the hamster antibody production (HAP) assay, and the rat antibody production (RAP) assay). The test sample is inoculated into

mice, hamsters, or rats, respectively. The animals are then housed for 28 days, after which sera are collected for testing using an enzyme-linked immunosorbent assay or immunofluorescent antibody-staining assay to determine if the animals have generated an immune response against any of the listed viruses. If no immune responses are detected, the test sample is considered free of those viruses. The detection of LCMV requires an additional step to be performed. If animals become infected with this virus early in life, a tolerance to the virus is developed, which results in no immune response to the virus if infected later in life. If such tolerant mice are used in the assay, they will not yield a positive immune response in the event that the test sample contains LCMV. To ensure that the animals used in the study are susceptible, they are inoculated with LCMV. If the animals are susceptible, a clinical pathology will be observed; should no clinical pathology be observed, the test fails as these animals have tolerance to the virus.

There is some criticism of the antibody production tests in the industry. To date, there have appeared in the literature no data on the validation of this assay; therefore the specificity and sensitivity have not been characterized. Alternative methods using PCR assays are available for those who seek to reduce the numbers of animals used in these studies. Care should be taken when attempting to use alternative assays to the antibody production tests. Although there are empirical data {[18] and references therein} showing that PCR testing is equivalent or superior in sensitivity to the antibody production assays, Q5A(R1) should be followed in this regard unless specific agreement is obtained from regulatory authorities.

10.3.7 Nucleic Acid Amplification Techniques

In recent years, the introduction of PCR technology has revolutionized the ability to detect contaminating viruses that are undetectable using the cell- and animal-based assays. The technology was first introduced in the late 1980s, and the detection of viruses was one of the first areas to exploit this. This simple technique enables the amplification of small amounts of target nucleic acid, such as a region of virus genome, to a level that it is easily detected. This allows any virus for which suitable primers are developed to be targeted and identified in a matter of hours, to a sensitivity level approaching a few copies of virus. It is stated in Q5A(R1) that "If the cell line used for production is of human or non-human primate origin, additional tests for human viruses, such as those causing immunodeficiency diseases and hepatitis, should be performed unless otherwise justified. The polymerase chain reaction (PCR) may be appropriate for detection of sequences of these human viruses as well as for other specific viruses" [1]. In practice, human and nonhuman primate cells are evaluated using an even greater panel of PCR tests, as shown in Table 10.5.

Table 10.5 Panels of PCR tests recommended for different production cell types.

Production cell	Recommended PCR tests
Human	Hepatitis A, B, and C viruses, enteroviruses such as human norovirus, human immunodeficiency virus, human T-cell lymphotropic virus, human circoviruses, parvovirus B19, human papillomaviruses, human polyomaviruses, human adenoviruses, Epstein–Barr virus, human cytomegalovirus, and human herpesviruses 6, 7, and 8
Nonhuman primate	Simian polyomaviruses, simian foamy virus, simian immunodeficiency virus, simian retrovirus, simian T-cell lymphotropic virus

10.3.8 Next-Generation Sequencing

Advances in the detection of viruses since the issuance of Q5A(R1) in 1997 have yielded new methods that may be useful additions to the panel of tests currently applied to cell line characterization. One of these is next-generation sequencing (NGS), otherwise referred to as massively parallel sequencing or deep sequencing. The advantage of NGS is that it represents a means of monitoring an MCB or CAL sample for all known viruses [19]. This technology involves the sequencing of all of the nucleic acids in a sample, such as a cell bank or bulk harvest. The detected sequences are then used to challenge a database of all of the known virus sequences to discover if any of the sequences match a specific virus. Currently, the technology is most likely to be applied to the characterization of novel vaccine cell substrates [20, 21] or production cell lines with potential gaps in their development histories. Barriers to more routine use of the technology include (i) the high cost, (ii) the lack of sufficient sequence information in public databases for some families or species of virus, and (iii) the possibility of discovering unrecognized sequences that may or may not represent a viral contaminant. The latter can present the manufacturer and the regulators with problems in interpretation. The technology may therefore need some adaptation before going into routine service. As mentioned previously, there is an expectation by the US FDA that analysis using NGS be presented in the case of novel cell substrates proposed to be used for vaccine production.

10.4 Qualification of Animal-Derived Raw Materials

In addition to the production cell itself, any other animal-derived materials that may be used in the upstream or downstream manufacturing processes represent potential introduction points for viruses. The first aspect of the safety triangle (Figure 10.1) therefore includes the qualification of any animal-derived

raw materials. Historically, the most commonly employed animal-derived materials have been bovine serum and porcine trypsin. Less frequently used materials have included bovine thrombin, bovine albumin, and others. An approach to mitigating the risk associated with the use of these materials is the reduction or elimination of animal-derived materials in the production of the biologic product. The use of defined serum-free growth media and the use of recombinant trypsin, thrombin, and albumin are examples of this.

Bovine serum has been implicated in contamination of manufacturing processes with epizootic hemorrhagic disease virus [22], vesivirus 2117 [6, 7], reovirus type 2 [8], and Cache Valley virus [8]. In cases where bovine serum cannot be eliminated from the upstream process, mitigation of risk involves three approaches: (i) sourcing strategies, (ii) implementation of a gamma irradiation step for inactivation of potential viral contaminants, and (iii) testing. Sourcing means procuring serum from reputable vendors who conduct release testing on their serum and can trace the geographical origin of the serum pools used. Gamma irradiation at 25–40 kGy will mitigate the risk of many of the most common viral contaminants (especially Cache Valley virus, epizootic hemorrhagic disease virus, and reovirus). Testing involves the 9 CFR 113.47 method discussed previously [15]. This amounts to the testing in Vero and a bovine cell such as BT cells of ~200 ml from one container of serum and explains why a negative outcome in this testing provides little assurance that the entire lot is free of contaminating virus.

The risk associated with the use of porcine trypsin can also be mitigated using the three approaches used for bovine sera. These involve sourcing; viral inactivation by UV treatment, gamma irradiation, or electron beam treatment; and testing. Porcine trypsin is also tested with the 9 CFR 113.47 method [15]. Typically the trypsin is neutralized and then applied to Vero cells and a porcine cell such as LLC-PK1. Again, a relatively small amount from one container is tested, and the assumption that the entire lot is safe on the basis of the result obtained is not entirely warranted.

10.5 Testing of In-Process and Lot-Release Samples

During the manufacture of biologicals, the upstream process typically involves the expansion of production cells from a vial of the WCB to final bioreactor volumes as great as 10,000 l. During this expansion, there is the risk that a contaminating virus may enter the product stream from a variety of sources (raw materials, environment, operators, etc.). In addition, there is the possibility that endogenous or latent viruses may be activated and amplified during the expansion of cells for production. The Q5A(R1) guidance therefore discusses the need for testing for viruses in unprocessed bulk. This constitutes the second aspect of the safety triangle (Figure 10.1).

Table 10.6 Viral detection tests performed on "unprocessed bulk."

Test	Frequency
All cell types:	
Electron microscopy for retroviral enumeration	First three lots
In vitro virus screening assay	All lots
CHO production cell:	
Specific detection assay for mouse minute virus	All lots
Specific detection assay for vesivirus	All lots

The guidelines suggest that the ideal time point to collect samples to monitor for virus is after the fermentation period has completed, and prior to any purification steps. This is considered to be the optimal point to detect the possible presence of contaminating viruses. In some instances, the unprocessed bulk harvest is not a single collection but is collected over a period of time and eventually pooled. The virus testing required for the unprocessed bulk is focused on the detection of adventitious viruses, including any specific viral contaminants that historically have been detected for a given production cell type (Table 10.6).

Transmission electron microscopy is expected to be performed on at least the first three lots of unprocessed bulk harvest material to estimate the total number of retrovirus particles per milliliter of sample. From this estimate, the virus load to the downstream purification is estimated, and the virus clearance capacity of the system that is needed to remove or inactivate this retroviral load with an adequate safety margin may be calculated (see also Chapter 14).

The *in vitro* screen for adventitious virus contamination is the primary test applied to the unprocessed bulk for routine viral safety. This assay is similar to that used for testing cell banks, but in this case the test material is a sample of the bulk harvest material. The *in vitro* assay normally is 14 days in duration (28 days for a human production cell) and uses both a cytopathic effect and hemadsorption end point. Other tests can be added for the bulk harvest on the basis of risk assessment. For example, a number of manufacturers using CHO production cells have added PCR or infectivity assays for known viruses of concern such as MMV or vesivirus on the basis of historical contamination events involving these viruses (Table 10.6). These may be implemented both as in-process assays (for business risk) and as lot-release assays (for regulatory compliance).

As expressed in Q5A(R1), bulk harvest materials in which adventitious viruses have been detected are not to be used for manufacturing drug. Under these circumstances, an investigation is expected to determine the root cause

and modifications to the manufacturing process and/or to testing strategies that are typically expected to prevent and facilitate detection of subsequent contamination events.

10.6 Viral Clearance (Removal and Inactivation) Studies

The third aspect of the viral safety triangle for biologics (Figure 10.1) is the characterization of the downstream purification process (DSP) for capability and capacity for viral clearance (removal or inactivation). Viral clearance studies are performed for two main reasons: (i) to provide evidence that the DSP is effective for the inactivation or removal of endogenous viruses (especially retroviruses) and (ii) to provide indirect evidence that the DSP might inactivate or remove adventitious virus contaminants. Endogenous viruses represent those viruses that are present in the production cell genome, while adventitious viruses are those that enter the production process from sources other than the production cell (i.e., other raw materials, culture media, operators, or the environment). The rationale for this testing is stated in Q5A(R1): "The goal should be to obtain the best reasonable assurance that the product is free of virus contamination" [1]. It cannot be assumed that qualification and testing of raw materials and testing of unprocessed bulk provides adequate assurance of viral safety. Demonstration of viral clearance by the DSP also is a necessary aspect of such assurance.

Viral clearance studies are both time consuming and costly, but are always necessary for obtaining approval for clinical studies and marketing authorization. Inadequate design or conduct of viral validation studies and lack of understanding of the issues surrounding these studies are frequent stumbling blocks in the approval of biologicals. Due to the handling of high titer viruses and the complexity of the experimental design, these studies are frequently outsourced to specialized contract testing laboratories.

The initial considerations for the scope of a viral clearance study are the inherent virus risks associated with the production cells, other raw materials, and the production process. The manufacturer should include a number of recognized viral inactivation or removal steps when designing the DSP (Table 10.7). Orthogonal virus removal steps are generally expected for regulatory approval. A process relying on a single clearance step such as filtration or partitioning by chromatography has less chance of success. Another common mistake made by manufacturers is to include results from two or more apparently different steps that in fact utilize the same mechanism of virus removal or inactivation.

A typical virus clearance study evaluates between four and six purification steps, each appropriately scaled down and demonstrated to be representative of full scale. Per Q5A(R1), each step is to be evaluated in a stepwise fashion so

Table 10.7 Downstream purification steps with viral clearance capability.

Purification step	Comments	Effectiveness
Chromatography	Many different types are available including protein A and other affinity, ion exchange, size exclusion, etc.	More effective with enveloped viruses as they tend to bind to anion exchange resins due to net negative charge. These approaches are not typically considered robust
Nanofiltration	Used frequently as a final step in the process. Some filters are able to exclude particles down to 15 nm	Predictable and robust step to use for small parvoviruses. Challenging to validate due to aggregates in the virus spike
Heat treatment	Pasteurization is long recognized as an effective treatment for bacteria but is also useful with some viruses. There is a disadvantage as some proteins are heat labile	Viruses vary in resistance to heat. Aggregation of product can be an issue during or following heat treatment
Low pH step	Commonly used step in combination with chromatography as a "hold" step. pH lower than 3.0 is rarely used.	Effective against enveloped viruses around pH 3.5. At this pH, retroviruses are rapidly inactivated. Herpesviruses are inactivated, but more slowly, and inactivation may be incomplete. Above pH 4, results are much more variable
Solvent or detergent	Many different detergents and solvents have been used to disrupt viral proteins. There is the possibility of damaging the product with such treatment	Effective only for enveloped viruses; the lipid content of the process stream can influence efficacy of treatment; the process stream should be free of gross aggregates. Following treatment there is the need to ensure efficient removal of the solvent or detergent
Precipitation	Selective removal of the product using precipitation is not a robust step and is not frequently used	Polyethylene glycol, ethanol, and octanoic acid have all been used in the past. Difficult to show effective clearance if the virus coprecipitates with the product

that the contribution of the individual steps can be determined. In addition, the mechanism of clearance (i.e., removal or inactivation) should be determined, where possible. For inactivation steps, it is expected that the time kinetics of inactivation be determined. The number of model viruses used to challenge each of these steps varies from two (early clinical stage) to four or more (late clinical stage and marketed drug). The viruses are chosen to cover,

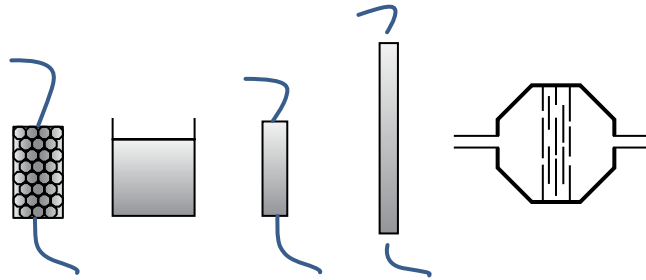
Table 10.8 Particle size ranges for model viruses from different families.

	Small (<50 nm)	**Large (>50 nm)**
Enveloped virus	*Flaviviridae, Togaviridae*	*Herpesviridae, Retroviridae, Paramyxoviridae, Rhabdoviridae, Poxviridae, Orthomyxoviridae*
Non-enveloped virus	*Parvoviridae, Picornaviridae*	*Adenoviridae, Reoviridae*

as widely as possible, the likely viruses that could contaminate the process (including both endogenous and adventitious viruses). In addition, the model viruses should cover, as much as possible, a wide range of physical characteristics (especially size and envelope status). Most studies therefore include enveloped and non-enveloped viruses of small and large size (Table 10.8).

The challenge virus stocks, sometimes termed spikes, are available from specialized service laboratories as purified stocks that have been well characterized with regard to characteristics such as titer, identity, and protein content. The viruses vary with respect to the titers that are routinely attained. Parvovirus stocks, for example, can be prepared at over 10^{12} infectious viruses per mL. Retroviruses, on the other hand, can typically be prepared at titers of around 10^{8} infectious viruses per mL. Determination of the amount of virus (titer) present in the spike and at the end of a process step is performed using cell-based infectivity assays or PCR assays. The latter cannot distinguish between inactivated and infectious virus, so it is used primarily to determine the removal of virus. An example of a typical virus clearance study for the production of a monoclonal antibody product is shown in Figure 10.3. It is expected that such studies be performed in duplicate (i.e., two independent studies). The use of appropriate controls for each study, and the conduct of toxicity and interference studies, to rule out product stream effects on the cell-based or PCR end points used to determine log reduction values, is also discussed in Q5A(R1).

The total amount of virus removed or inactivated by the DSP is calculated by adding the $\log_{10}$ reduction values for those steps with values greater than 1. The overall clearance value obtained for the model retrovirus can be compared to the amount of retrovirus that may be present in unprocessed bulk at the end of the fermentation process. The assay used to estimate the amount of retrovirus in the bulk is typically the TEM, as discussed previously. Per Q5A(R1), the DSP "should be able to eliminate substantially more virus than is estimated to be present in a single-dose-equivalent of unprocessed bulk." In the example previously mentioned, the DSP has the capacity to clear 10^{14} $\log_{10}$ of retrovirus. Typical TEM counts in a bulk harvest would be around 10^{4}/ml. For such a process, there would be 10 $\log_{10}$ of excess clearance capacity for retroviruses (see also Chapter 14).



Model virus	Purification step				
	Protein A affinity	Low pH hold	Size exclusion	Ion exchange	Nanofilter
Retrovirus	2[a]	>5	2	0	>5
Herpesvirus	2	4	0	0	>5
Parvovirus	<1	<2	0	0	>6
Adenovirus	<2	<2	0	0	>6

[a]Virus $\log_{10}$ reduction determined for each step.

Figure 10.3 An example of a viral clearance study. Five steps are investigated: one inactivation step (low pH) and four removal steps. Four model viruses are used (retrovirus, herpesvirus, parvovirus, and adenovirus).

At present, each manufacturing processes requires a specific validation study to be completed. The clearance characterization must be repeated should there be any significant change to the manufacturing process. Although not mentioned in Q5A(R1), generic (platform) validation studies have been discussed widely in the industry [23]. In a generic validation, a DSP step such as nanofiltration using a particular filter under specific parameters of use would be validated for virus reduction. A similar process for a different product that employed the same filter under equivalent parameters would be able to claim the same effective log reduction. That is, the nanofiltration would not have to be validated specifically for the new product. In practice, such platform or generic validation is rarely invoked.

10.7 Summary

The ICH Q5A(R1) guidance provides a systematic approach for evaluating and mitigating the viral risks associated with biotechnology product manufacture. The safety triangle shown in Figure 10.1 encapsulates the expectations expressed in the guidance. Each aspect of the triangle is viewed as necessary for assuring the safety of biotechnology products. Figure 10.4 summarizes the

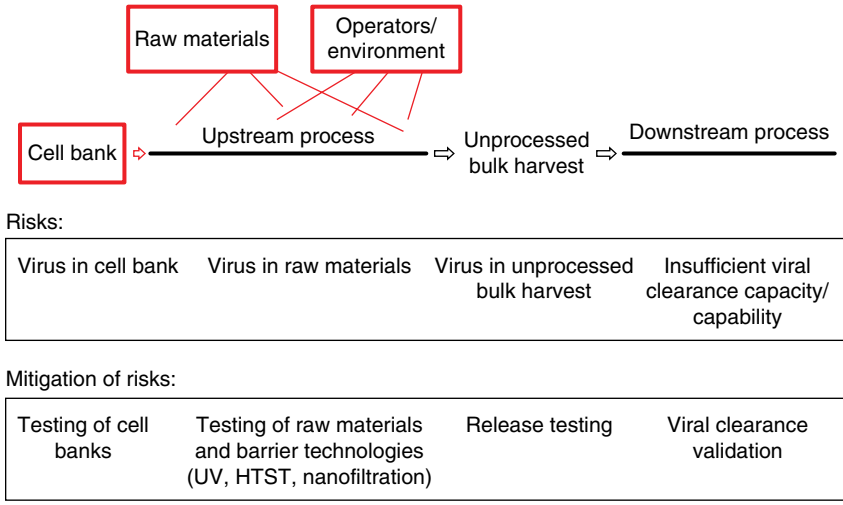


Figure 10.4 ICH Q5A(R1) in practice. Virus entry points are displayed in boxes. The primary threats are those impacting the upstream (cell culture process). Operator and environmental threats are best mitigated through the use of closed processes. HTST, high temperature/ short time treatment; UV, ultraviolet irradiation. The downstream process includes viral clearance steps.

implementation of Q5A(R1) in practice. The most common entry points for viruses are shown relative to the entire production process, and the risk mitigation strategies that are expected to be put in place are shown.

The Q5A(R1) guidance is a little dated, relative to the ongoing improvements in assay methodologies for detection of viral contaminants. Experts in the viral safety arena, the current author included, call for the revision of the guidance in the following respects: (i) the elimination of the requirement for performing the *in vivo* virus screen and (ii) the replacement of the antibody production assays with the appropriate PCR panels. These changes reflect the opinion that animal testing should be minimized where possible, the empirical data demonstrate equivalence or superiority of the PCR methods as compared with the antibody production methods [18], and the fact that the *in vivo* method has contributed little to biologics safety in the past [16].

The risks inherent in a biotechnology manufacturing process are not always fully apparent, and the industry continually learns from the revelations of viral contamination incidents such as those reported by Genzyme [6]. As a result of such revelations, regulatory expectations may change, and testing strategies may need to change accordingly. New analytical technologies may provide increased assurance of the viral safety of biologics in the future.

References

1 International Conference on Harmonisation (ICH) 1997. Q5A(R1) Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin.
2 European Pharmacopoeia. Chapter 5.1.7 Viral Safety. 01/2008/50107.
3 Garnick RL 1996. Experience with viral contamination in cell culture. *Dev Biol Stand* 88:49–56.
4 Moody M, Alves W, Varghese J, Khan F 2011. Mouse minute virus (MMV) contamination—a case study: detection, root cause determination, and corrective actions. *PDA J Pharm Sci Technol* 65:580–588.
5 Skrine J 2011. A biotech production facility contamination case study—mouse minute virus. *PDA J Pharm Sci Technol* 65:599–611.
6 Qiu Y, Jones N, Busch M, Pan P, Keegan J, Zhou W, Plavsic M, Hayes M, McPherson JM, Edmunds T, Zhang K, Mattaliano RJ 2013. Identification and quantitation of vesivirus 2117 particles in bioreactor fluids from infected Chinese hamster ovary cell cultures. *Biotechnol Bioeng* 110:1342–1353.
7 Oehmig A, Büttner M, Weiland F, Werz W, Bergemann K, Pfaff E 2003. Identification of a calicivirus isolate of unknown origin. *J Gen Virol* 84:2837–2845.
8 Nims R 2011. Adventitious viral contamination of biopharmaceuticals: who is at risk? *BioProcess J* 10:4–10.
9 Hughes JV, Messner K, Burnham M, Patel D, White EM 1996. Validation of retroviral detection for rodent cell-derived products and gene therapy applications. *Dev Biol Stand* 88:297–304.
10 Lander MR, Chattopadhyay SK 1984. A Mus dunni cell line that lacks sequences closely related to endogenous murine leukemia viruses and can be infected by ecotropic, amphotropic, xenotropic, and mink cell focus-forming viruses. *J Virol* 52:695–698.
11 Shepherd AJ, Wilson NJ, Smith KT 2003. Characterisation of endogenous retrovirus in rodent cell lines used for production of biologicals. *Biologicals* 31:251–260.
12 Lovatt A, Black J, Galbraith D, Doherty I, Moran MW, Shepherd AJ, Griffen A, Bailey A, Wilson N, Smith KT 1999. High throughput detection of retrovirus-associated reverse transcriptase using an improved fluorescent product enhanced reverse transcriptase assay and its comparison to conventional detection methods. *J Virol Methods* 82:185–200.
13 US FDA 1993. Points to consider in the characterization of cell lines used to produce biologicals. FDA/CBER.
14 Code of Federal Regulations (CFR) 1993. Detection of cytopathogenic and/or hemadsorbing agents. 9 CFR 113.46.
15 Code of Federal Regulations (CFR) 1995. Detection of extraneous viruses by the fluorescent antibody techniques. 9 CFR 113.47.

16 Sheets RL 2013. Opinion on adventitious agents testing for vaccines: why do we worry so much about adventitious agents in vaccines. *Vaccine* 31:2791–2795.

17 Gombold J, Karakasidis S, Niksa P, Podczasy J, Neumann K, Richardson J, Sane N, Johnson-Leva R, Randolph V, Sadoff J, Minor P, Schmidt A, Duncan P, Sheets RL 2014. Systematic evaluation of *in vitro* and *in vivo* adventitious virus assays for the detection of viral contamination of cell banks and biological products. *Vaccine* 32:2916–2926.

18 Mahabir E, Brielmeier M, Schmidt J 2007. Microbiological control of murine viruses in biological materials: methodology and comparative sensitivity. A review. *Scand J Lab Anim Sci* 34:47–58.

19 Barzon L, Lavezzo E, Militello V, Toppo S, Palù G 2011. Applications of next-generation sequencing technologies to diagnostic virology. *Int J Mol Sci* 12:7861–7884.

20 Ma H, Galvin TA, Glasner DR, Shaheduzzaman S, Khan AS 2014. Identification of a novel rhabdovirus in *Spodoptera frugiperda* cell lines. *J Virol* 88:6576–6585.

21 Shabram P, Kolman JL 2014. Evaluation of A549 as a new vaccine cell substrate: digging deeper with massively parallel sequencing. *PDA J Pharm Sci Technol* 68:639–650.

22 Rabenau H, Ohlinger V, Anderson J, Selb B, Cinatl J, Wolf W, Frost J, Mellor P, Doerr HW 1993. Contamination of genetically engineered CHO-cells by epizootic haemorrhagic disease virus (EHDV). *Biologicals* 21:207–214.

23 Brorson K., Krejci S., Lee K., Hamilton E, Stein K, Xu Y 2003. Bracketed generic inactivation of rodent retroviruses by low pH treatment for monoclonal antibodies and recombinant proteins. *Biotechnol Bioeng* 82:321–329.

11

ICH Q5B Analysis of the Expression Construct in Cell Lines Used for Production of Recombinant DNA-Derived Protein Products

Jianxin Ye[1,2], Zhong Liu[1,3], and David Pollard[1,2]

[1] *Merck & Co. Inc., Kenilworth, NJ, USA*
[2] *Currently at: Amicus Therapeutics, Cranbury, NJ, USA*
[3] *Currently at: Adello Biologics, Piscataway, NJ, USA*

11.1 Introduction

International Conference on Harmonisation Q5B [1] is a guidance document addressing the characterization of the expression constructs introduced into eukaryotic or prokaryotic cells to be used for production of recombinant DNA (r-DNA)-derived protein products. This guidance was finalized under step 5 in July 1996 and implemented by the European Union in December 1995, by the US FDA in February 1996 and by Japan's MHLW in January 1998.

The Q5B guidance provides the rationale for the analysis of expression constructs and describes the types of information that should be supplied (i.e., are considered valuable) regarding expression construct characterization. It is also made clear that the guidance is not intended to cover the entire quality aspects of r-DNA-derived protein products, as these may be addressed in other ICH documents, such as Q5A(R1) [2] or Q5D [3]. Analysis of the expression construct, as described in more detail herein, is considered to be part of the overall quality evaluation of a protein product, along with characterization of the structural features and efficacy of the recombinant protein itself.

For preparation of regulatory filings, the information discussed in this chapter should be included in Common Technical Document (CTD), section 3.2.S.2.3 *Control of Materials*.

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11.2 Expression Construct

Per Q5B, the term "expression construct" refers to "the expression vector containing the coding sequence of the recombinant protein" [1]. In addition to the coding sequences for the recombinant protein, the expression construct also contains the necessary genetic elements to facilitate the expression of the recombinant protein by the production cell. These include the promoters, enhancers, polyadenylation signals, origins of replication, antibiotic-resistant genes, and selection markers. Where the recombinant protein consists of multiple different peptides, as is the case for monoclonal antibodies (mAb), the DNA sequences encoding the different peptides may be located on a single vector or on separate vectors.

The purpose of analyzing the expression construct, per Q5B, is "to establish that the correct coding sequence of the product has been incorporated into the host cell and is maintained during culture to the end of production" [1]. The key words here are "correct" and "maintained." After the expression construct is introduced into a host cell, either as integrated copies in the chromosome or as extrachromosomal elements, the coding sequences for the recombinant protein may undergo mutations. Although such mutations are expected to occur at an extremely low rate, these undesired events could potentially alter the properties of the encoded protein product.

Various protein analytical techniques are routinely expected to be used for product characterization. These techniques can be used to confirm the primary and higher-order structures of the expressed protein, identify any post-translational modifications, and determine product heterogeneity. Because different analytical techniques assess only a specific product attribute, the Q5B guidance indicates that "no single experimental approach can be expected to detect all possible modifications to a protein" [1]. In this regard, orthogonal methods are often needed at both protein and nucleic acid levels to assure the correct primary structure of an expressed protein product. Nucleic acid sequencing, for example, confirms the correct DNA or mRNA sequences coding for the protein, as opposed to the protein amino acid sequence itself.

Nucleic acid analysis may be performed on DNA or mRNA/cDNA depending on the situation. In cases where multiple copies of the expression construct are integrated into the production cell chromosome, some of these copies might not be transcriptionally active. In such instances mRNA/cDNA analysis might be more appropriate than examination of genomic DNA. In cases of extrachromosomal expression, plasmid DNA isolated directly from the cell line represents the most appropriate subject for such analysis. Verification of the coding sequences is not intended to identify all possible mutations. The methods also may not cover variants with low abundance. Rather they are intended to verify that the genetic information encoding the primary protein structure is correct.

Another critical aspect of the rationale for characterizing the expression construct is to ensure that the correct coding sequence of the product "is maintained during culture to the end of production." This is an important element of "genetic stability." The genetic stability data should also be provided along with the characterization data demonstrating correctness of the coding sequence(s). This will be further discussed in Section 11.3.

It is expected, per ICH Q5B, that the analytical methodologies employed for these characterization assays be validated, including estimates for the limit of detection of low abundance sequence variants.

11.3 Characterization of the Expression System

Section III of Q5B "characterisation of the expression system" describes the types of information that should be supplied in regulatory filings to support the characterization of the expression construct. The section includes three subsections: (i) expression construct and cell clone used to develop the master cell bank (MCB), (ii) cell bank system, and (iii) limit for *in vitro* cell age for production.

In the following sections, a mAb product will be used as an example to illustrate the satisfaction of expectations of the ICH Q5B guidance. Similar expectations for testing and methodologies typically employed apply for other types of r-DNA proteins. This testing may be performed by the product manufacturer if such capabilities exist within the organization or may be outsourced to a variety of contract testing organizations having the requisite expertise.

11.3.1 Expression Constructs and Cell Clone Used to Develop the Master Cell Bank (MCB)

11.3.1.1 Origin of the Nucleotide Sequences Coding for the Protein

Per ICH Q5B, the manufacturer should provide information regarding the origin of the nucleic acid sequence(s) introduced into the production cell. This might include the source organism of the cell from which the sequence was originally isolated and any sequence modifications. For example, for a typical mAb product, generation of the coding sequences for the light chain and heavy chain peptides may involve animal immunization/hybridoma generation (or library screening), identification of the parental cDNA sequences, and any subsequent protein engineering, such as humanization. Description of these steps is expected. For instance, the source of the immunogen, breed of the animals immunized, sequences of the parental antibody, germline types of the human Ig backbone used for humanization, and any amino acid sequence modifications to the complementarity-determining region and to the rest of the heavy and light chain peptides must be described. As codon optimization is currently a common practice to facilitate protein expression by the production cell, the resulting DNA coding sequences post codon optimization should also be

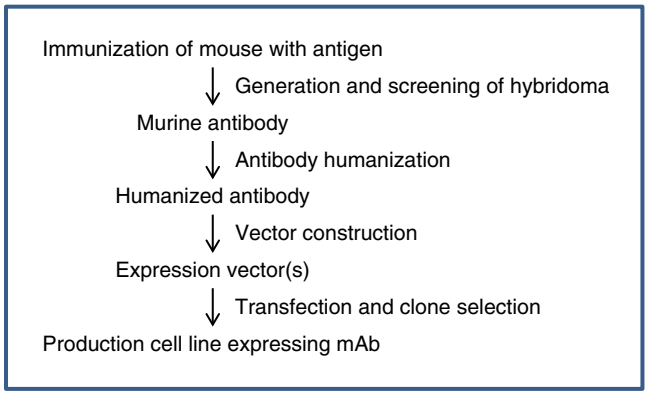


Figure 11.1 Overall strategy for the development of a mAb-expressing production cell.

provided. A schema depicting, at a high level, the steps typically taken during generation of a mAb-producing cell is illustrated in Figure 11.1.

11.3.1.2 The Assembly of the Expression Construct

To properly express the target recombinant protein, the DNA coding sequences need to be assembled into an appropriate expression vector. One or multiple DNA cloning steps are usually involved in this process. It is expected, per Q5B, that a detailed description of these cloning steps and the restriction endonucleases involved in the digestion processes will be provided. The information about the source vectors should also be provided.

To illustrate the final expression vector(s), a detailed component map and a complete annotated sequence of the plasmid should be supplied. For example, the enhancers/promoters used to drive the expression of the recombinant protein, the polyadenylation signals, the origins of replication, the antibiotic-resistance genes, the selection markers, etc., must be displayed. All expressed proteins, including the product of interest and selection markers, should be indicated on the map. The fully assembled expression vector should be subjected to DNA sequencing to confirm that the coding sequence (including the junction of insertions) of the recombinant protein is correct. If the protein expression involves other elements such as introns or flanking sequences, those regions should be sequenced as well. For the remainder of the expression construct, it should be indicated whether the sequences have been derived from literature or confirmed by sequencing. Figure 11.2 shows a schematic component map of an expression vector for a mAb.

11.3.1.3 Generation of the Production Cell Clone

Once the final expression vector is generated, it is then transfected into the host cell to generate production cell clones. A detailed description of the methods used for introducing the expression construct into the host cell is expected.

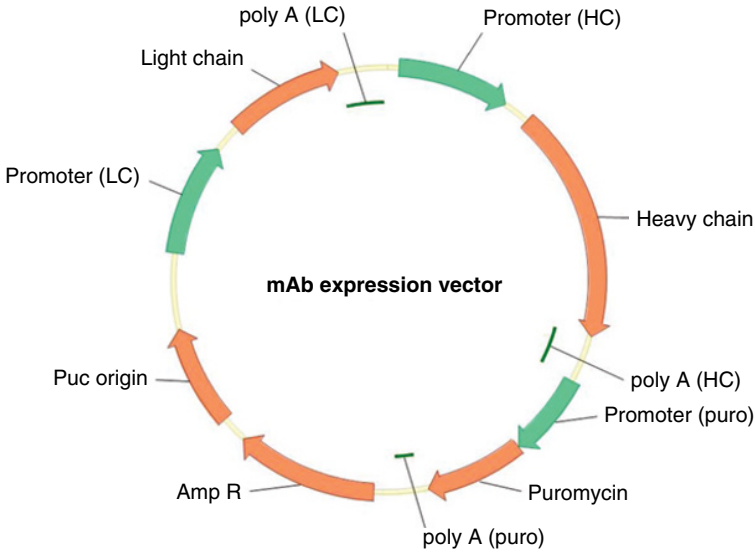


Figure 11.2 A schematic component map for a mAb expression vector. Amp R, ampicillin resistance; HC, heavy chain; LC, light chain; mAb, monoclonal antibody; poly A, polyadenylation; Puc, plasmid cloning vector pUC; puro, puromycin.

Utilization of animal-sourced materials should be avoided if possible, and any that are used should be fully documented. If DNA amplification is involved, the methods used for amplification should also be provided in detail. Typical production cell line generation activities such as cell line screening, selection of clones, and subcloning should also be described in detail.

As a common practice, multiple candidates of cell clones are generated as described earlier and are evaluated before a final manufacturing clone is selected (see also Chapter 13). During the evaluation (screening process), the correct protein sequence should be confirmed on both the protein and nucleic acid levels (Table 11.1). Due to the sensitivity limitations of sequencing methods, there is a possibility of having low levels of mutant contamination in the DNA used for transfection. In addition, mutations can also take place during transfection. These can ultimately result in mutations in the expression construct, which is integrated into the host genome. To mitigate the risk of selecting clones with an incorrect sequence, it is a good practice to maintain multiple clones or transfected cell pools prior to subcloning.

Furthermore, the protein primary structure and nucleic acid sequence should be confirmed before the final manufacturing clone is selected for cell banking (Table 11.1). The expectation for the aforementioned information on cell line generation per ICH Q5B is consistent with the ICH Q5D guidance (see Chapter 13), which specifies that information on the cell substrates be reported.

Table 11.1 Summary of testing expectations per ICH Q5B.

Purpose	Expectation	Endpoint	Typical method
Characterization of production clone	Evaluation of the expressed protein	Amino acid sequence	Peptide mapping, LC-MS
		Nucleic acid sequence	Direct sequencing of mRNA or cDNA
Characterization of MCB and WCB	Analysis of the expression construct integrated chromosomally	Coding sequence	Direct sequencing of mRNA or cDNA
		Analysis of integration sites	Restriction endonuclease mapping/Southern blot analysis
		Analysis of gene deletions/insertions	
		Copy number	Quantitative PCR
	Analysis of extrachromosomal expression constructs	Coding sequence	Direct sequencing of plasmid
		Percent of production cell population containing the construct	Plasmid loss assay
Characterization of CAL	Analysis of the expression construct (integrated)	Coding sequence	Direct sequencing of mRNA or cDNA
		Analysis of integration sites	Restriction endonuclease mapping/Southern blot analysis
		Analysis of gene deletions/insertions	
		Copy number	Quantitative PCR

For recombinant protein products, the final cell clone should be derived from a single cell progenitor. This monoclonality is intended to assure that the protein product has the correct sequence by having a homogeneous production cell population. Monoclonality also implies a reproducible product, so it is important to the regulatory agencies. The methods used to demonstrate monoclonality and the results obtained should therefore be discussed.

11.3.2 Cell Bank System

A two-tiered bank system is usually used for the production of recombinant proteins. This is discussed in more detail in Q5A(R1) (see Chapter 10) and Q5D (see Chapter 13). The cell bank system is comprised of a MCB and one or more working cell banks (WCB). The MCB is generally derived from the selected final manufacturing clone carrying the expression construct and represents multiple aliquots prepared from the same cell pool and stored

appropriately. As discussed earlier, the coding sequences for the recombinant protein should have been confirmed before creation of the MCB.

The WCB is derived by expansion of one or multiple ampoules of the MCB. In practice, for early clinical phases, the production of clinical materials may be derived from the MCB, since only a limited number of batches are required at this stage. In such cases, a WCB might not yet have been generated.

All cell banks are expected to be characterized using appropriate tests to determine their represented phenotypic or genotypic characteristics and the absence of adventitious agents (testing requirements are described in more detail in Chapter 13). The results of analysis of the expression construct in the cell banks should also be provided.

The analysis of the expression construct in the banked cell includes both the coding sequences and the physical state of the expression constructs (Table 11.1). For production cells in which the expression construct has been integrated into the chromosomes, the preferable approach for coding sequence confirmation involves direct sequencing of the mRNA or cDNA. Restriction endonuclease mapping/Southern blotting may be used for the analysis of integration sites and potential gene deletions/insertions. To increase the confidence in the results of this analysis, a few sets of restriction enzymes or enzyme combinations are generally used to generate a specific digestion pattern. For gene copy number analysis, real-time polymerase chain reaction (PCR) (also referred to as quantitative PCR) is commonly used. With recent advances in next-generation sequencing (NGS) technologies, it is foreseeable that this method could potentially be applied for these purposes and NGS may eventually replace some of the traditional techniques used for the characterization of the constructs, especially the physical state of the expression constructs [4]. For production cells in which the construct is located extrachromosomally, the expression construct should be isolated directly from the banked cells without subcloning. The construct may then be characterized by nucleic acid analysis. This is done in order to ensure that the analysis is representative of the banked cell population. Furthermore, the percentage of production cells retaining the expression construct should be determined (Table 11.1).

11.3.3 Limit for *In Vitro* Cell Age for Production

As discussed earlier, the two key words in the purpose set forth in ICH Q5B "to establish that the correct coding sequence of the product has been incorporated into the host cell and is maintained during culture to the end of production" [1] are "correct" and "maintained." This section focuses on the expectations intended by "maintained."

For evaluating cells at the limit of *in vitro* age (CAL), production cells derived from an aliquot of the WCB are expanded beyond the end of the typical (pilot- or full-scale) production process. Cells harvested at this point are then used for

the evaluation (these are sometimes referred to as "end of production cells" (EOPC)). The expression construct analysis used for evaluating the MCB should be performed, including the confirmation of the correct coding sequence. The CAL nucleic acid analysis is not required for each production run, but should be performed at least once for each MCB.

In the case of a process change that leads to a longer time period from WCB vial thaw to the end of production, data should be provided to justify the extended *in vitro* cell age limit. This typically involves repeating the CAL analysis using the new process conditions.

11.4 Conclusions

The characterization of the expression construct of the production cell line and the evaluation of the protein product generated by r-DNA biotechnological manufacturing processes are each important for assuring the quality of a recombinant protein product. The types of information that are expected within regulatory submissions are described in ICH Q5B, and the philosophies underlying the cloning and cell banking of the engineered cell are addressed both in this guidance and in other ICH documents, particularly ICH Q5D (discussed in Chapter 13). These guidance documents should therefore be referred to early during production cell design and construction, so that the various required data will be captured and documented in sufficient detail to satisfy the articulated expectations. In this chapter, we have used the creation of a mAb production cell as an example of the implementation of the expectations of ICH Q5B, including examples of the types of analyses that are commonly employed to satisfy these expectations.

References

1 International Conference on Harmonisation (ICH). Q5B. Analysis of the expression construct in cell lines used for production of r-DNA derived protein products. 1996.
2 International Conference on Harmonisation (ICH). Q5A(R1). Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin. 1997.
3 International Conference on Harmonisation (ICH). Q5D. Derivation and characterisation of cell substrates used for production of biotechnological/biological products. 1998.
4 Metzker M 2010. Sequencing technologies—the next generation. *Nature Rev Genet* 11:31–46.

12

ICH Q5C Stability Testing of Biotechnological/ Biological Products

John G. Davies[1], Di Gao[2], Yoen Joo Kim[1], Richard Harris[2], Patricia W. Cash[1], Timothy L. Schofield[1,3], Roujian Zhang[2], and Qiang Qin[2,]*

** Corresponding author*
[1] MedImmune, Gaithersburg, MD, USA
[2] AstraZeneca, Frederick, MD, USA
[3] Current Affiliation: GlaxoSmithKline, Rockville, MD, USA

12.1 Introduction

The ICH Q5C guideline [1] was released in 1995 to supplement guideline Q1A(R2) [2] by specifically addressing the unique characteristics of biological drugs compared with small molecule drugs. The document covers a wide range of well-characterized proteins and polypeptides, either purified from natural sources or manufactured from recombinant DNA technology. The guidance also applies to both conventional and recombinant vaccines. In the past two decades, the biotechnology industry has brought many new biological therapeutics and vaccines into the market. Biological products are therefore becoming an even more integral part of the product portfolio for both traditional "pharmaceutical" companies and biotechnology companies.

Biological products are more complex than their small molecule counterparts because they are derived from complicated biological processes that are subject to both environmental and genetic variability. The final products are typically a mixture of product variants that have slightly different structures but identical or similar biological activities. Some of these variants are the products of post-translational modifications (e.g., glycosylation), while some are the products of degradation during manufacturing and storage (e.g., deamidation). These modifications can be combinational and may impact the stability of the molecule in a reiterative and synergistic way. This may make stability modeling, data extrapolation, and shelf-life prediction more challenging for biological macromolecule drugs than for synthetic small molecule drugs. Due to these

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complexities, Q5C makes it very clear that for biologicals, "long-term, real-time, real-condition" stability studies are required to establish the requested storage period. Experimental designs and protocols for stability studies should take into account this mandate. Nevertheless, as will be described in the following text, Q5C still offers the potential for case-by-case examination and the possibility to utilize other data (e.g., extrapolation of shelf life using accelerated stability data).

Q5C is relatively clear in terms of the logistics for a typical stability study. This chapter will focus on the typical questions/ambiguities encountered during the design and conduct of such stability studies.

12.2 Purposes of Stability Studies

Different types of stability studies are mentioned in both the Q1A(R2) and Q5C guidelines. Different storage conditions are expected to be used in these studies. The studies and the storage conditions evaluated serve multiple purposes at different stages of product development and the product life cycle. It is critical that the goals of each stability study and its design be understood. Depending on the purpose of the studies, some must be performed in the representative containers at recommended storage condition and under a GMP environment, while others may not need to have such restrictions.

12.2.1 Analytical Method Development

At the very early stages of product development, a suite of analytical methods is developed to facilitate initial process and formulation development. Stress stability or forced degradation studies are performed at this stage to generate an increasing amount and number of degradants to aid in development of analytical methods. A subset of analytical methods should be stability-indicating to ensure that all degradants can be monitored and controlled during manufacturing and storage. Such studies are typically conducted by the analytical and formulation research and development functions of the company. Platform knowledge of the common degradation pathways and critical quality attributes for the particular molecule can guide the method development efforts. Compared with relatively homogeneous small molecule drugs, biological drugs are typically a heterogeneous mixture of structurally related variants such as size and charge variants, different glycoforms, and so on. The analytical methods to be used should be able to quantify such variants.

12.2.2 Formulation Development

One widely adopted approach of formulation development is to subject the product in different formulations to various stability/stress conditions. The formulations that demonstrate the best stability under various conditions can

then be further evaluated for final formulation selection. A great deal of knowledge and experience has been gained in this field in the past two decades [3].

12.2.3 Degradation Pathway Elucidation

Stability studies, especially accelerated and stress stability studies, can be used to elucidate degradation pathways for the product being evaluated. Biologics and vaccines are manufactured and formulated for maximum stability under the recommended storage conditions, and degradants are not typically observed at these storage conditions. Under accelerated and stress conditions, degradation is accelerated and degradants can be monitored at earlier time points and in greater quantity.

Degradants that appear during recommended storage and accelerated conditions should be characterized and their criticality should be evaluated. If such degradants could potentially impact the safety and efficacy of the product, control strategies should be implemented and in-process or release specifications for the specific degradants may be required. Sometimes such degradants exist only in trace amounts under recommended storage conditions. Stress studies are used to generate higher amounts of these degradants in order to facilitate the elucidation of their identity (structure). For degradants that appear only under stress conditions, it is still beneficial to understand the nature (mechanism) of the degradation. However, it is sometimes challenging to completely characterize the degradation pathways due to complexity of the degradation (e.g., after photostress). This is true for small molecules and even more so for large biological molecules. ICH Q1A(R2) suggests that "it may not be necessary to examine specifically for certain degradation products if it has been demonstrated that they are not formed under accelerated or long term storage conditions" [2].

12.2.4 Expiry (Use-By Date) Establishment

The shelf life of drug substance and final drug product should be established primarily based on "long-term, real-time, real-condition" stability studies. Stability samples should be obtained from representative manufacturing processes and should be stored in representative containers (scaled down if needed). At the end of the proposed shelf life, the drug substance and drug product must be shown to meet predetermined specifications. Such stability studies are typically performed following GMP procedures. Any unexpected, out-of-trend, or out-of-specification results should be adequately investigated following the established internal quality systems of the testing organization and of the sponsor of the study. Such an investigation might seek to establish whether the aberrant result is due to laboratory error, method variability, or failure of the test material to meet the specified quality requirements.

For intermediates (e.g., purification pools created during production), allowable hold times and hold conditions should also be established based on stability studies using representative materials and conditions. These may be performed in the development lab instead of in a quality control setting.

Degradation of biological products is a multifaceted, complicated process, and the typical Arrhenius equation may not apply for a particular degradation pathway. Alternatively, the Arrhenius analysis may apply for one attribute, but not for others. While the Q5C guideline is explicit in that real-time, real-condition (temperature, container) stability data are required to establish expiry, certain agencies may be more open than others to use accelerated/stress stability data to extend expiry. A comprehensive data package (such as demonstration of Arrhenius behavior) will help justify such extrapolation.

12.2.5 Storage/Transportation/In-Use Condition Excursions

During manufacturing, handling, transportation, and in-use in clinics, drug substance or drug product may sometimes be subjected for short periods of time to conditions that are outside of the recommended storage conditions. For example, a vial of liquid monoclonal antibody product can be left at room temperature inadvertently for a day rather than being stored in the refrigerator as specified. Has the drug's safety and efficacy been compromised? Stability studies conducted under accelerated conditions, including higher than optimal temperature and relative humidity (as appropriate) as well as multiple freeze–thaw cycles, can provide the data needed to justify the acceptability of drug substance or drug product subjected to an occasional excursion in storage conditions.

12.2.6 Comparability/Similarity

Stability studies are an indispensable component of a comparability exercise (see also Chapter 15 on ICH Q5E [4]). For any two products (either the same product before and after a process change or a biosimilar product vs. innovator) to be considered comparable, their stability profiles should be comparable as well. The assumption is that any degradation pattern or rate difference observed could represent a difference in the products under consideration that may or may not be detected through other comparability approaches.

For products such as monoclonal antibodies, typical accelerated (e.g., 25°C) and stress conditions (37 or 40°C) may not be harsh enough to degrade the products. In this case, harsher forced degradation conditions (e.g., 60 or 10°C below the product melting temperature) might be used. Although there is a common tendency/expectation to degrade the products to 10–20% relative to the intact protein, the ability and necessity to achieve this is highly dependent on the particular attribute being investigated and the capability of the analytical method. For a precise method (e.g., size-exclusion chromatography (SEC)),

a linear, consistent degradation rate over time can be demonstrated despite a limited extent of degradation. As long as the degradation rates can be measured accurately, such results are still relevant to demonstrate comparability.

Since the goal of such degradation studies is to demonstrate comparability and these data are not used to establish or extrapolate expiry, different storage conditions and containers than the ones used in typical stability studies may be used. For example, to establish the shelf life for a lyophilized drug product, the stability study must be done using product in the lyophilized state stored in the actual/representative drug product containers. However, to demonstrate comparability before and after a process change, the lyophilized drugs can be reconstituted in a buffer and transferred from the original containers (e.g., vials) into different containers to facilitate quick comparison of the degradation profiles and rates under the same conditions for the two comparator products. Such studies can be performed in much shorter time (e.g., 2–3 weeks) in a development lab than the typical accelerated and stress stability studies that might take 3–6 months in a GMP quality control lab.

12.3 Degradation Pathways of Therapeutic Proteins

As therapeutic proteins constitute the most significant class of biopharmaceuticals, this section is focused on the most common degradation pathways observed in these molecules. Vaccine degradation mechanisms have been described in a recent review [5].

Proteins undergo various degradation pathways during manufacturing, storage, transportation, and handling by end users. At typical recommended storage temperatures (e.g., 2–8°C) and conditions (e.g., protected from light), the rates of degradation are typically low, and it may take months or years before any change is noticed. The most commonly observed protein degradation pathways are aggregation, fragmentation, oxidation, deamidation/isomerization, disulfide bond scrambling/exchange, glycation, and desialylation.

12.3.1 Aggregation

Aggregation is one of the most commonly observed degradation pathways for a therapeutic protein [6–9]. Aggregation results when a monomer molecule associates with other monomers through covalent or noncovalent interactions to form dimers, trimers, multimers, or high molecular weight species. The aggregates may be formed either reversibly or irreversibly. Aggregation may lead to the formation of subvisible or visible particles. For therapeutic proteins, both aggregates and particles pose a safety concern due to their potential immunogenicity.

Protein aggregation occurs due to various causes and under various circumstances, such as incorrect folding during protein expression, perturbation of the native conformation during purification, formulation, freeze–thawing, freeze drying, ultrafiltration/diafiltration, vial or syringe filling, pumping, transportation, or storage.

An increase in aggregate/particle formation is frequently observed in stability studies. The analytical methods that are most commonly used to detect protein aggregation are SEC, analytical ultracentrifugation (AUC), dynamic or static light scattering, and asymmetric flow field flow fractionation (AFFF or AF4). Typically, it is a good practice to verify that the SEC method does not have any resin–analyte interactions by using an orthogonal method (e.g., AUC) that is performed in solution free of any resin. Once the lack of interaction is verified, it is not necessary to run the orthogonal method throughout the stability study.

12.3.2 Fragmentation

Fragmentation refers to the disruption of peptide (amide) bonds within a protein as a result of chemical or enzymatic reactions. Heat, certain pH, free radicals, trace metals, and light can all facilitate protein fragmentation [10]. Peptide bonds between certain amino acids (e.g., those between aspartic acid and proline residues) are also points of potential degradation, being inherently susceptible to chemical hydrolysis [11]. Residual proteases (e.g., cathepsin family) that have been reported to exist in many biologics can result in fragmentation of the biologic under conditions favoring protease activity [12, 13].

The hinge region of monoclonal antibodies (Figure 12.1) is another "hotspot" for fragmentation. Fragments generated by cleavage in this region can often lead to well-resolved smaller fragments with a larger complementary fragment that is partially resolved from the main peak [15–17].

Commonly used methods for detecting and quantifying fragments include SEC either under native or denatured conditions, reduced and nonreduced sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and the capillary versions (CE-SDS) and reversed-phase high performance liquid chromatography (RP-HPLC).

12.3.3 Oxidation

Heat, light, trace metals, and oxygen radicals can each oxidize susceptible amino acids, especially methionine, tryptophan, and histidine. Methionine is the amino acid residue most susceptible to oxidation. The impact of protein oxidation is highly dependent on the location of the amino acid residues being oxidized. For residues located in the complementarity-determining region (CDR), oxidation can potentially impact the affinity of the monoclonal for the

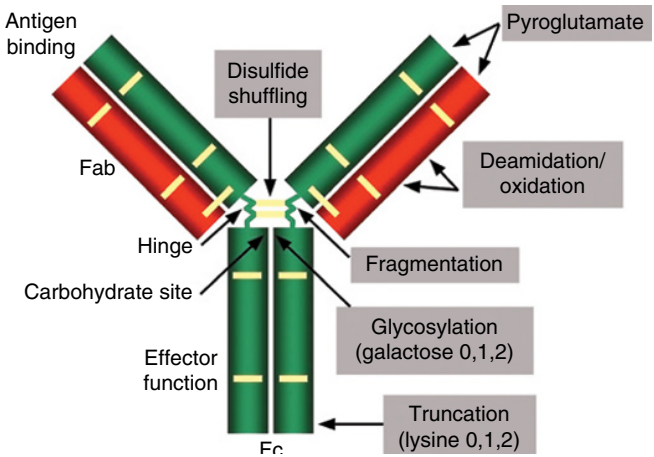


Figure 12.1 Structural features of a therapeutic antibody. *Source*: Chirino 2004 [14]. Reproduced with permission of Nature Publishing Group.

antigen, impacting the product's biological activity. It is well known that oxidation of methionine residues in the Fc region impacts the affinity of the monoclonal antibody for the neonatal Fc receptor (FcRn), therefore potentially impacting the serum half life of the antibody [18–20].

Tryptophan oxidation is also commonly observed during stability studies, especially in photostability studies. The tryptophan degradation pathway is complicated and can often lead to multiple degradants and can cause discoloration of the therapeutic protein [21, 22].

Polysorbate used in protein formulations can degrade in the presence of light or metals and can induce subsequent protein oxidation.

Oxidation of a biologic can be monitored by RP-HPLC peptide mapping with or without mass spectrometry (MS). Methionine oxidation in the Fc region of a monoclonal antibody (Figure 12.1) can also be measured by protein A affinity chromatography. Good resolution of the oxidized species of an antibody has also been achieved using a mixed-mode (i.e., separation by both size and hydrophobicity) column [23].

Protein oxidation has been reported to affect a variety of protein properties, including binding affinity to antigen, effector functions, and serum half life. Because of this, oxidation is considered one of the major concerns in therapeutic monoclonal antibody development. Strategies that may be used to prevent undesirable oxidation of monoclonal antibody drug products include use of appropriate secondary packaging and addition to the formulation of additives minimizing oxygen and reactive oxygen species (e.g., including methionine or EDTA as scavengers of free radicals and metals) [24].

12.3.4 Deamidation/Isomerization

Protein deamidation refers to the loss of an amide functional group from asparagine (Asn) or glutamine (Gln) residues. Subsequent hydrolysis of the succinimide intermediate yields a hydroxyl group, forming aspartic acid (and isoaspartic acid) or glutamic acid residues. Protein sequences that are most susceptible to deamidation are those in which asparagine/glutamine residues are followed immediately by members of the glycine (Gly) group (Asn/Gln-Gly) [25, 26]. Figure 12.2 illustrates the deamidation pathway of the Asn residue.

Deamidation is a commonly observed protein degradation pathway. In addition to the susceptibility due to the protein's intrinsic structure previously mentioned, the deamidation rate depends on pH, temperature, ionic strength, and buffer type.

Deamidation is typically favored at high pH, while isomerization can happen under acidic conditions. The impact of deamidation on the biological activity of the therapeutic protein is highly dependent on the location of the

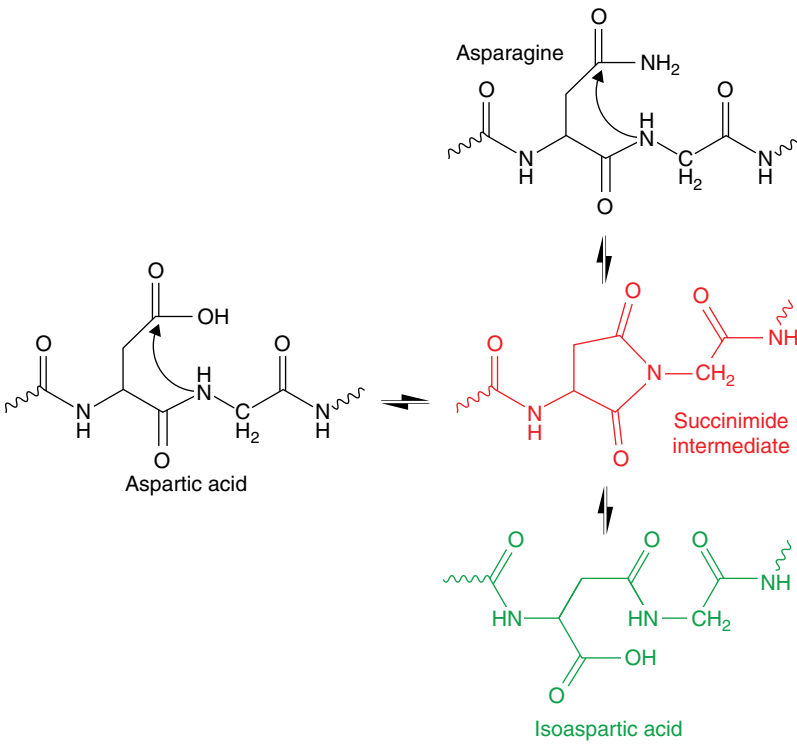


Figure 12.2 Representative scheme of Asn deamidation pathway. (Figure is from Wikipedia [27].)

modification. For example, deamidation/isomerization in the CDR of monoclonal antibodies has been reported to impact potency [28], while deamidation in the Fc region of the monoclonal antibody molecule is generally considered to have much less impact [29].

12.3.5 Disulfide Bond Scrambling/Exchange

Disulfide bonds play a critical role in maintaining the structural integrity of therapeutic proteins. Monoclonal antibodies contain multiple intra-chain and inter-chain disulfide bonds. For antibodies of the IgG_2 subclass, it is known that various disulfide isoforms coexist [30, 31]. Some cysteines in the protein backbone may exist in the reduced form in which a free thiol group is available for reaction. Such free thiols may induce disulfide bond scrambling and induce protein aggregation [32].

12.3.6 Glycation

Glycation occurs when a protein is incubated in the presence of a reducing sugar, such as fructose or glucose. This is also referred to as nonenzymatic glycosylation. The amine group of an amino acid, typically lysine (Lys), reacts with the carbonyl group of the reducing sugar and results in covalent bonding [33]. Glucose and, occasionally, other reducing sugars (e.g., galactose) are present in culture media, and glycation can occur during the upstream cell culture process. Reducing sugars are not typically present in final drug substance or product formulations. However, nonreducing sugars such as sucrose or trehalose are widely used as stabilizers/bulk agents in formulations. These nonreducing sugars can undergo hydrolysis during storage and can generate reducing sugars that can react with proteins, resulting over time in glycated product.

Boronate affinity chromatography can be used to assess protein glycation through the affinity of *cis*-diol bearing sugar moieties to phenylboronate chromatography resins. More recently, MS of intact proteins has been used to estimate the overall extent of glycation, and peptide mapping coupled with tandem mass spectrometry (MS/MS) can provide detailed characterization of specific glycation sites [34].

12.3.7 Desialylation (for Glycoproteins)

Sialic acid or neuraminic acid is a nine-carbon monosaccharide that is widely present in mammalian glycoproteins. For glycoprotein therapeutics (e.g., erythropoietin), sialic acid plays a critical role in biological activity and/or in *in vivo* half life. Loss of sialic acid can occur slowly during storage under stability studies. Glycan mapping can be used to monitor sialic acid loss.

Table 12.1 summarizes the common degradation pathways observed in therapeutic proteins and corresponding analytical methods.

Table 12.1 Common degradation pathways for therapeutic proteins and associated analytical methods.

Physical/chemical degradation	Accelerating conditions	Commonly used methods	Additional characterization methods
Aggregation	Thermal, light, shear, agitation, pH	SEC, CE-SDS (nonreduced)	AUC, AFFF
Fragmentation	Thermal, light, free radicals, pH	SEC, CE-SDS (reduced)	RP-HPLC, mass spectrometry
Oxidation	Thermal, light, oxidizer, metal, peroxide	Peptide mapping, protein A affinity chromatography	Mass spectrometry
Deamidation	High pH	cIEF, IEX	Chromatofocusing, mass spectrometry
Desialylation	Thermal	cIEF, IEX, sialic acid analysis	Glycan mapping
Glycation	Thermal, pH	cIEF, IEX	Boronate affinity chromatography, mass spectrometry
Disulfide scrambling	Thermal, pH, metal	Ellman, peptide mapping	RP-HPLC, IEX

AFFF, asymmetric flow field flow fractionation; AUC, analytical ultracentrifugation; CE-SDS, capillary sodium dodecyl sulfate-polyacrylamide gel electrophoresis; cIEF, capillary immunoelectrophoresis; IEX, ion-exchange chromatography; RP-HPLC, reversed-phase high performance liquid chromatography; SEC, size-exclusion chromatography.

12.4 Stability Testing Protocol

Release and stability testing are a critical part of the overall control strategy employed to demonstrate the quality, purity, safety, and potency of biological products. In comparison with small molecules, degradation of biological/biotechnological products can be complex and may follow various pathways, including chemical degradation (oxidation/deamidation/glycation, etc.), fragmentation, aggregation, changes to higher-order structure, and interactions with container/closure components or excipients.

At the time of submission of a marketing application for a new biological entity, the routes of degradation for the molecule should be well understood. Knowledge of the degradation pathways/profiles can be derived from stability studies performed under forced degradation conditions, including both accelerated and stress conditions, which are conducted as part of the formal stability program during product development. This information may also be obtained by specific forced degradation studies targeting chemical modification of the

molecule. A comprehensive and robust stability protocol should include testing designed to detect major routes of degradation specific to the molecule and methods to monitor biological activity and safety.

12.4.1 Stability-Indicating Profile

ICH Q5C recommends that "on the whole, there is no single stability-indicating assay or parameter that profiles the stability characteristics of a biotechnological/biological product. Consequently, the manufacturer should propose a stability-indicating profile that provides assurance that changes in the identity, purity and potency of the product will be detected" [1]. Stability-indicating parameters should be those that might be expected to change over the shelf life of a drug substance or drug product, which may have impact on patient safety or efficacy.

Due to the number of attributes that might be tested, care should be taken to select only those that are impactful. A risk assessment should be undertaken to select attributes and a design that gives meaningful information regarding product quality over the entire shelf life and to limit the burden of non-value-added testing as well as the risk and associated effort in investigating false positive or out-of-trend/out-of-specification results.

12.4.2 Assay Selection

12.4.2.1 Physicochemical Integrity

Typically, the purity of a biological product is determined by more than one method. Methods should be selected that are capable of detecting changes that may result from chemical degradation, aggregation, and fragmentation. Analytical methods routinely employed to monitor purity include high resolution chromatography methods (SEC, reversed-phase chromatography, ion-exchange chromatography), gel electrophoretic techniques (reducing and nonreducing gel electrophoresis, isoelectric focusing), and peptide mapping. Some of the methods are more stability-indicating than others and should be given priority when designing a stability protocol. For a typical monoclonal antibody molecule, SEC and charge-based methods (ion-exchange chromatography or isoelectric focusing) are the methods that have been found most sensitive for detecting changes.

12.4.2.2 Biological Activity

Potency testing measures the ability of a product to achieve its intended biological effect and is a critical part of any stability protocol for biological products. The potency method (or methods) selected and included in the marketing application should be relevant to the mechanism of action (MOA) of the product or at least a surrogate assay that has been bridged to an assay that reflects MOA. For some biologics (e.g., bi-specific T-cell engagers (BiTEs))

that have a complex MOA, a single bioassay may not be sufficient, and multiple potency assays may be required to provide sufficient measurement of biological activity.

The three main types of methods for measuring biological activity include binding methods, cell-based bioassays, and animal-based assays. Binding methods (e.g., enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance) provide a measure of an antibody–antigen interaction. If employed in isolation, these methods are suitable only for products where the MOA is solely determined by ligand binding. Cell-based bioassays measure a defined biological effect *in vitro* (the exact mechanism being specific to the molecule in question). Animal-based methods measure the biological effect *in vivo* but are rarely used for routine lot release or stability assays due to the challenges in performing such testing. Typically *in vivo* bioassays are expensive, lengthy to perform, and often associated with high levels of assay variability.

Potency test methods should include either an international reference standard or a qualified in-house reference standard if an international standard does not exist. The potency results obtained for a test sample should be reported relative to the reference standard activity. While bioassays should be included in stability protocols for therapeutic proteins and vaccines, these may not represent very sensitive stability-indicating methods: (i) biologicals such as monoclonal antibodies are relatively stable under recommended storage conditions, and during their proposed shelf lives, their potencies may not change significantly; and (ii) certain degradation pathways expected to occur during storage in stability studies that can be detected by more sensitive assays may not impact potency. Despite these considerations, regulatory agencies expect to see evidence demonstrating that the bioassay is stability-indicating to ensure the assay can monitor potency changes. This can be achieved through a focused forced degradation study in which product undergoes severe degradation that is not typically observed at the recommended storage conditions.

During the life cycle of the product, as more and more data are accumulated during continuous process validation, the removal or reduction of bioassay testing during stability studies may be justified.

12.4.2.3 Quantity (Assay)

For proteins, quantity is typically determined by UV absorbance at 280 nm using a theoretical or experimentally derived extinction coefficient. The quantity or content of the protein drug product should and typically does remain unchanged during the course of stability studies. However, there are factors that may cause the apparent protein content to change: (i) leachable compounds from containers/closures that cause an increase in UV absorbance at 280 nm; (ii) evaporation occurring during the stability study if container/

closure integrity is compromised; (iii) generation of degradants that have higher UV absorbance at 280 nm than the protein itself (e.g., byproducts of Maillard reactions) [35]; (iv) loss of protein in solution due to adherence to the inner surface of the primary container (this can only be observed with very low concentration products); and (v) microbial contamination.

Certain additives or excipients in the formulation (e.g., polysorbate) may be critical to product stability. If there is any indication that degradation of such materials may adversely affect the quality of the drug product, this degradation may need to be monitored during stability studies.

12.4.2.4 Safety

It is critical that sterile drug products remain sterile during their shelf lives. Sterility testing should be performed as part of drug product stability testing to demonstrate the safety of the product throughout its expiry period. Per current FDA guidance [36], sterility testing should be performed during drug product release (as container/closure integrity testing cannot demonstrate initial sterility), but at subsequent stability time points, container/closure integrity testing can be performed in lieu of sterility testing. European authorities have provided similar scientific guidance: "sterility testing should be performed at least at the end of shelf life but it can be replaced by testing of the container closure integrity" [37]. Commonly used container/closure integrity methods include the widely adopted yet less sensitive methods such as dye ingress, vacuum decay, and microbial challenge, as well as newer technologies such as high-voltage leak detection and laser-based gas headspace analysis.

Safety testing may also be considered for drug substance stability protocols. If the drug substance stability protocol utilizes reduced-size containers (otherwise representative in terms of product contact materials but not necessarily filled under the same conditions as the actual drug substance in GMP manufacturing), then a more suitable approach may be to develop a control strategy around testing the actual full-size containers that will be used to hold the drug substance at various operational hold points in the process. When drug substance is sampled from full-scale containers at each time point, inclusion of safety testing in the drug substance stability protocol is a valid approach to assuring the material remains within acceptable limits over its shelf life. Commonly used safety tests for drug substance include bioburden, sterility, and endotoxin.

12.4.2.5 Other Product Characteristics

Visual appearance, pH, and subvisible particulates should be monitored during stability testing. For lyophilized drug products, reconstitution time and moisture content should also be included. Moisture change may also need to be considered if permeable or semipermeable container/closures are to be used.

A visual appearance test should constitute a measure of color and clarity and an assessment of visible particulates. Changes in visual appearance during stability studies can indicate that a significant change in product quality has occurred. For biologics/biotechnological products in particular, the control of visible/subvisible particle levels is especially important because of the propensity of proteins to form particulates. This has been a hot topic with regulators, and a method for quantifying particle numbers as well as a means of differentiating between intrinsic (inherent to the product) and extrinsic (not inherent to the product) visible particles is desirable. Instrument-based methods for measuring color and clarity are available and can report results relative to international color/clarity reference materials. The primary method for quantifying visible particles remains visual inspection. This is a relatively subjective method that relies on adequate training of analysts.

Changes in pH occurring during stability are not typically expected for biological products. When this occurs, it may be indicative of excipient breakdown or of leachables from interaction of the product with the container/closure. Changes in pH may lead to chemical degradation of the product.

Levels of subvisible particles for injectable products and parenteral infusions should be routinely monitored as part of drug product stability testing. The most common technique for quantitating the amount of subvisible particles greater than 10 μm in size is the light obscuration method. Subvisible particles of less than 10 μm in size have come under increased scrutiny by regulatory agencies in recent years due to concerns around possible immunogenicity [8, 38, 39]. While there is currently no requirement to set acceptance criteria around the smaller-sized subvisible particles (i.e., <10 μm), a method should be implemented to capture and report such data during drug product stability studies. At present, there is no universally accepted method for measuring levels of particles less than 10 μm in size.

For drug products contained in semipermeable container/closures, moisture loss testing should be performed. This can be carried out by monitoring container weight after storage under low humidity conditions. This is not a requirement for drug products stored in impermeable containers. Container/closure integrity testing can potentially be used to replace moisture loss testing during stability studies.

12.5 Stability Conditions and Time Points

12.5.1 Long-Term Storage Condition

The long-term storage condition represents the defined storage temperature at which the product will be stored (i.e., the storage condition that will appear on the container label). ICH Q5C defines the recommended testing frequency

at the long-term storage condition as illustrated in the following (this is similar to the recommendation made in ICH Q1A(R2)):

- When shelf lives of 1 year or less are proposed, the real-time stability time points should include monthly testing for the first 3 months and at 3-month intervals thereafter.
- For products with proposed shelf lives of greater than 1 year, the real-time stability time points should include testing every 3 months during the first year of storage, every 6 months during the second year, and annually thereafter [1].

12.5.2 Accelerated and Stress Storage Conditions on Formal Stability

In contrast to the ICH Q1A(R2) tripartite guideline, ICH Q5C does not make specific recommendations regarding intermediate and accelerated testing time points or define the conditions of accelerated and stress conditions (stating rather that these should be considered on a case-by-case basis for biological products). Q5C does, however, make a strong recommendation that accelerated and stress studies be performed on both drug substance and drug product.

Accelerated testing is defined by ICH Q1A(R2) as studies designed to increase the rate of chemical degradation or physical change of a drug substance or drug product by using exaggerated storage conditions as part of the formal stability studies. These are intended to provide valuable data that can be leveraged for many purposes as discussed in Section 12.2 of this chapter.

Examples of accelerated and stress conditions commonly employed in formal stability studies are illustrated in Table 12.2.

Note that the stress storage conditions listed in Table 12.2 represent examples of temperatures used in routine stability studies to demonstrate degradation of therapeutic proteins.

For some biological products, 25°C may be sufficient to demonstrate degradation, while for others, even 40°C may be insufficient. For example, lyophilized drug products typically demonstrate stability at these temperatures and may require higher temperatures or a longer exposure to 40°C to demonstrate detectable degradation. Conditions and time points should be selected on a

Table 12.2 Commonly used accelerated and stress conditions for biologics.

Long-term storage condition	Accelerated storage condition	Stress storage condition
−90 to −70°C, −50 to −30°C	2–8°C	23–27°C
2–8°C	23–27°C	38–42°C

case-by-case basis. While there is no formal requirement to include thermal stress conditions as part of routine stability, it is recommended that these conditions be applied to some stability studies early in clinical development to support the analytical method selection for real-time stability studies. Thermal stress conditions also provide value for stability throughout clinical development and into the commercial stability phase. Thermal degradation data from multiple lots allow for more accurate understanding and modeling of lot-to-lot variability within a process and provide greater confidence in use of thermal stability as a tool for demonstrating comparability.

12.5.3 Other Stress Testing

In addition to the inclusion of a thermal stress condition for routine stability, additional stress testing should be performed (described in ICH Q1A(R2) section 2.1.2). While the actual conditions selected are usually established on a case-by-case basis, stress conditions for a biological product typically include thermal stress, extreme pH exposure, oxidation (chemical), shaking/shearing, multiple freeze–thaw steps, and photolysis. The light conditions described in ICH Q1B [40] are typically too harsh for biologicals and incur severe complicated degradations. Milder, more relevant light conditions that mimic manufacturing and healthcare facility settings are more meaningful for understanding the light sensitivity of a product during manufacturing, storage, and clinical/commercial use.

12.5.4 Batch Selection

At the clinical stage of product development, at least one batch representative of the manufacturing process of the material to be used in clinical trials should be evaluated. The typical industry practice is to place a batch of representative development material (usually derived from the toxicology batch) on stability, in addition to the first clinical batch. Additional product batches may be put onto stability in the event of manufacturing process changes, or in support of materials where deviations might have occurred during the manufacturing process and/or batch release testing has demonstrated out-of-trend (yet still within specification) results, or simply to gather more information on lot-to-lot variability. During late-stage clinical development where manufacturing process and product presentation is consistent with or similar to that proposed for commercial stage, it is beneficial to increase the number of lots on stability to at least three in support of shelf life in the marketing application if no major changes are to be made during later development.

Within a marketing application, stability data should be provided for at least three representative batches of drug substance and drug product, and a minimum of 6 months of stability data should be included (for drug substance and

drug product with planned storage periods of less than 6 months, the minimum amount of data to be included is determined on a case-by-case basis). This is in contrast with ICH Q1A(R2), which specifies that a minimum of 12 months of stability data should be collected for small molecule drugs. The drug substance and drug product batches monitored should be representative of the manufacturing scale of production. Stability data derived from the pilot plant scale may, however, be provided in regulatory files with a commitment to place the first three manufacturing-scale batches into the long-term stability program. Pilot scale is defined as "the production of the drug substance or drug product by a procedure fully representative of and simulating that to be applied at manufacturing scale. The method of cell expansion, harvest, and product purification should be identical except for the scale of production" [1]. This allows for the possible use of clinical stability data to be leveraged to set initial commercial-scale shelf life (provided the clinical material falls within the definition of pilot scale and provided the storage containers and temperatures are identical to those for the commercial scale).

Stability data should be provided in support of all aspects of intended use of the material (e.g., if a drug substance may be held frozen or at 2–8°C, then stability data must be gathered in support of drug product produced using drug substance held at these conditions). Similarly, if multiple freeze–thaws of drug substance are to be allowed, stability data must be generated to support the manufacture of drug product produced from drug substance subjected to freeze–thaws. Stability data should also be provided in support of any proposed hold times for intermediates.

12.5.5 Sample Container/Closure

At all stages of product development, the drug substance placed on stability should be stored in containers representative of the actual storage containers used during manufacture. The use of reduced-size containers is permitted, provided that the contact materials and container/closure systems are identical to those used in manufacture.

Drug product with different container orientations should be included in the stability study to evaluate potential impact of protein/container interactions. Typically, vials are stored in both inverted and upright positions, while syringes are stored horizontally. Data should be collected for such orientations early in product development and potentially can be used to justify the use of worst-case scenarios only for later studies. Many biological drug products have multiple presentations in terms of concentrations, fill volumes, container sizes, and administration devices. A risk-based approach should be taken to design an efficient yet defendable stability plan to cover all of these presentations. ICH guideline Q1D [41] does allow a bracketing and matrix approach in the design of stability studies.

12.6 Stability/Shelf-Life Specification

A primary goal of stability evaluations is to assign and support the shelf life of investigational product and product to be marketed commercially. Shelf life is defined as the time period following manufacture during which the product is expected to remain within the approved shelf-life specification, provided that it is stored under conditions defined on the container label. For biological/biotechnological products, ICH Q5C specifically mandates that stability data for drug substance (bulk material) and drug product (final product) be included in the regulatory submission. A shelf life is not required to be assigned for intermediates (e.g., in-process pools), but data supporting the proposed hold times for such intermediates should be generated. Stability evaluation is performed and shelf life is established for drug substance in order to demonstrate that the bulk material can be stored at a predetermined storage condition for a specified duration without impact to the quality of the final drug product manufactured using this material.

Different approaches may be used to set shelf life at different stages of product development.

The European Medicines Agency (EMA) has described acceptable approaches for assigning a shelf life to a biological/biotechnological product during clinical trials, stating that "extension of the shelf-life beyond the period covered by real-time stability data may be acceptable, if supported and justified by relevant data" [42]. While it is recommended that shelf life be assigned using real-time stability data, allowance is made for alternate means of assigning shelf life during clinical trials. For instance, extrapolation is permitted within defined parameters (the maximum shelf life after extension should not exceed a twofold increase over the original shelf life and should not be more than 12 months beyond the real-time stability data available for representative batches). Any extrapolation of stability data assumes that any degradation pattern for the product is predictable and will continue to apply beyond the available real-time data. General statistical approaches for support of extrapolated shelf life are presented in ICH Q1E [43] section 2.6. An extrapolated shelf life for a product should not exceed the planned long-term sampling duration of the applicable stability protocol.

The EMA guideline also allows for prior knowledge (e.g., platform experience and knowledge of the manufacture of monoclonal antibodies) to be utilized in support of shelf life. While platform knowledge alone may not be sufficient to assign shelf life, it may be utilized as supporting data. Application of stability data from a previous manufacturing process (where applicable) can also be utilized as supporting data or in some cases can be used to assign shelf life to a material generated using a similar process. In the latter case, comparability (both of degradation pattern and rate) should be demonstrated.

Accelerated and stress testing may be leveraged for this approach, with the objective being to compare the degradation pattern and rates of the material manufactured using the new versus the original process. The accelerated degradation pattern is leveraged for the new process to demonstrate similarity of degradation products with the previous process, and the degradation rates can be assessed using an equivalence test to demonstrate that any difference in degradation rates falls within predetermined limits. The goal is to demonstrate that the stability determined for the existing process can be leveraged to assign shelf life for the material produced in the new process, rather than directly utilizing the accelerated data to extrapolate shelf life at the long-term condition for the new process. Any shelf life assigned using this approach should, of course, ultimately be verified with real-time stability studies performed on the product manufactured using the new process.

By the time of submission of a marketing application, the shelf life of biological/biotechnological products should be supported by real-time stability data, with a minimum of 6-month data available at time of submission as stated in ICH guideline Q5C. This is in direct contrast to guidance given in ICH Q1A(R2) and ICH Q1E, where an extrapolated approach to setting shelf life is permitted. One likely reason for this discrepancy is that ICH Q1A(R2) and ICH Q1E address small molecule drugs, where degradation pathways are relatively simple and typically follow the Arrhenius equation. Biological/biotechnological products, on the other hand, may be particularly sensitive to temperature, and the degradation observed under thermal challenges may not be representative of the degradation pathways at the long-term storage condition. Typically, small molecule degradation follows first-order kinetics, thus making it possible to extrapolate from accelerated data to the long-term storage condition. Biological/biotechnological product degradation may or may not follow first-order kinetics. Second-order or higher-order kinetics for biological product degradation may result from multiple interactions of the multiple forms of the molecule (a characteristic of complex biological molecules). These may present complications for subsequent stability data modeling. The decision tree for shelf-life evaluation presented in ICH Q1E Appendix 1, outlining considerations for applying accelerated data in the extrapolation of expiration dating, was written with small molecules in mind and may not be suitable for assessment of the stability of biological/biotechnological products.

Stability data generated for pilot-scale material can be used to support the shelf life proposed in a marketing application, as long as the pilot-scale material can be shown to be fully representative of the commercial-scale product, albeit at a reduced scale. Generally speaking, this provision opens the door for inclusion of stability data generated for late-stage clinical trial materials, with the caveat that the manufacturing process (other than scale) has not changed and that both container/closure and storage conditions remain unchanged.

Comparability for both degradation pattern and rates should be demonstrated between the pilot-scale material and the commercial-scale material in this case. A minimum of three lots of materials from each process scale should be evaluated in such comparability studies.

Typically, regulatory agencies still expect real-time recommended storage condition shelf-life stability studies for biologicals at the time of licensure application. However, when sound scientific justification, comprehensive data sets, and appropriate risk analyses are provided, agencies may be open to shelf-life data extrapolation from accelerated conditions on a case-by-case basis.

12.7 Approaches Used to Determine Shelf-Life Data for the Marketing Application

Setting shelf-life stability specifications has been a challenging topic for both the industry and regulatory authorities. Patient safety and drug efficacy should always be the top factors to consider when setting stability specifications. Due to the complexity of biological molecules and their degradation patterns, however, it is rare that a particular molecular quality attribute can be linked to the safety profile of the drug. A combination of process capability, manufacturing data, clinical exposure experience, criticality of the quality attributes (impact on safety and efficacy), and analytical method capability are taken into consideration when setting stability specifications. Regulatory agencies typically prefer that sponsors take a conservative approach intended to protect the patient rather than reducing business risk. A risk/benefit analysis should be performed to justify the proposed shelf life and associated specifications. The following hypothetical example provides a case study for the typical approach to establish shelf life and associated stability and release specifications.

Statistical approaches for shelf-life estimation are detailed in ICH Q1E [43]. Examples of the analyses that may be performed on stability data are shown in the case study in the following text.

12.7.1 Case Study for Establishing Stability Specifications

Three lots of drug product are to be placed on long-term stability. The minimum stability specification for product purity is 96.0%, which can be justified using clinical experience. The hypothetical data set is illustrated in Table 12.3. Note that the data illustrated in Table 12.3 are reported to three decimal places (five significant figures). In most cases, the values should be reported with the same precision used in the associated specification. For the purpose of data analysis and modeling, it is often worthwhile to use unrounded data to reduce variability.

Plots of the long-term stability data for purity for each lot on stability are shown in Figure 12.3.

Table 12.3 Long-term stability data for a hypothetical drug product held at the recommended storage condition.

Time point (months)	Product purity (%)		
	Lot 1	Lot 2	Lot 3
0	98.891	99.199	99.078
3	98.423	99.086	98.724
6	98.085	98.855	98.462
9	97.611	98.324	97.611
12	96.922	98.034	97.623
18	96.444	97.856	97.145
24	96.450	97.523	96.805
36	95.401	96.423	95.811

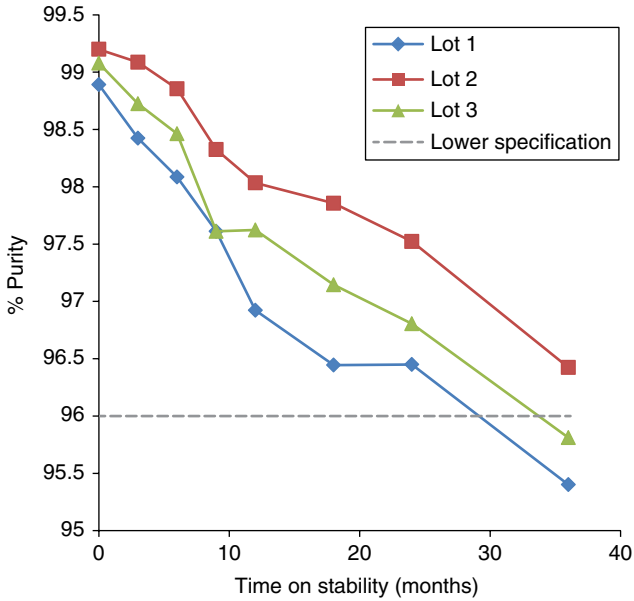


Figure 12.3 Plots of long-term stability data for three lots of a hypothetical drug product.

12.7.1.1 Calculation of Shelf Life by Individual Stability Measurements

The shelf life determined by the individual stability measurements is 24 months. Lots 1 and 3 fail the purity specification at the 36-month time point; therefore the shelf life is determined based on the last time point for which each of the lots passed, which is 24 months.

12.7.1.2 Calculation of Shelf Life Using Statistical Analysis

An alternative approach that might be used to determine the shelf life of this product on the basis of the data shown in Table 12.3 would be to use statistical analysis of the stability data following the outline illustrated in ICH Q1E. The more comprehensive analysis would be that using data from all three lots. Analysis of covariance (ANOCOVA) is performed to test the equality of slopes and intercepts among the lots for which data are available. The following possible outcomes are displayed in Table 12.4.

A statistical software package was employed to analyze the data illustrated in Table 12.3. The first step of the analysis is to establish whether a pooled slope can be used to model the data. The results of ANOCOVA testing the hypothesis of equal slopes for the three lots are shown in Table 12.5.

The source "Time*Lot" is used to test equality of the three % purity versus time slopes. An F-test statistic is calculated along with an associated significance probability [($P(>F)$]. The significance probability for equality of slopes was determined to be $P(>F) = 0.2711$ in this case. ICH Q1E recommends using a significance level of 0.25 to determine equality [43]. It was therefore concluded that the % purity versus time slopes could in this case be pooled, since the determined $P(>F)$ of $0.2711 > 0.25$.

A similar analysis is performed to establish the equality of the y-intercepts (estimates of the time_0 purity of the lots). The results of this ANOCOVA are given in Table 12.6.

Table 12.4 Possible outcomes for shelf-life determination from statistical analysis of data from three hypothetical product lots.

Slope equality	*y*-intercept equality	Shelf-life determination
Equal	Equal	Shelf life may be determined from pooled data
Equal	Unequal	Shelf life may be determined by applying pooled slope to the worst-case lot
Unequal	Equal/unequal	Shelf life should be determined from the worst-case lot

Table 12.5 ANOCOVA test of the hypothesis of equal slopes for % purity versus time for three hypothetical drug product lots.

Source	Degrees of freedom	Sum of squares	*F* statistic	*P*(>*F*)
Lot	2	3.1479	24.3431	<0.0001
Time	1	22.5434	348.6589	<0.0001
Time*Lot	2	0.1816	1.4046	***0.2711***

Table 12.6 ANOCOVA test of the hypothesis of equal *y*-intercepts for % purity versus time for three hypothetical drug product lots.

Source	Degrees of freedom	Sum of squares	*F* statistic	*P*(>*F*)
Lot	2	3.147918	23.3965	***<0.0001***
Time	1	22.54336	335.1018	<0.0001

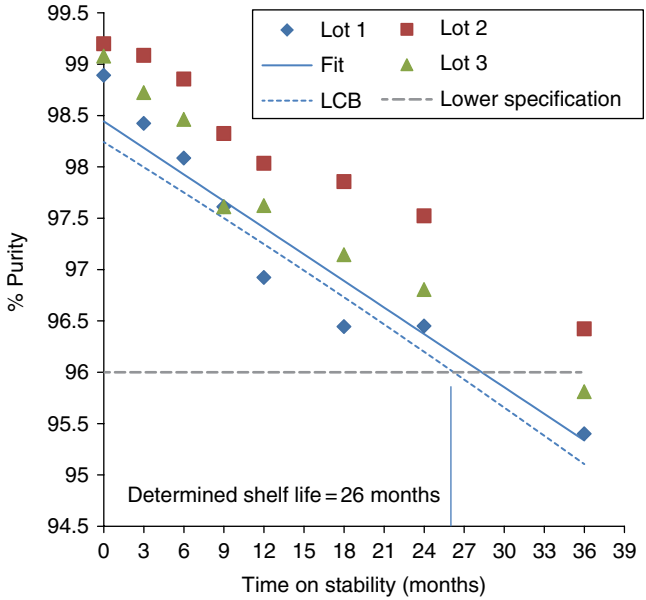


Figure 12.4 Linear regression analysis and application of the 90% confidence interval for three drug product lots.

The source "Lot" is used to test the equality of the three *y*-intercepts of the % purity versus time plots. The significance probability for equality of intercepts is *P*(>*F*), which was determined to be <0.0001. In this case, it was not concluded that the *y*-intercepts were equivalent since the *P*(>*F*) of <0.0001 < 0.25.

On the basis of the results shown in Tables 12.5 and 12.6, the shelf life for the hypothetical product must be determined using a pooled slope applied to the worst-case lot, as illustrated in Figure 12.4. The line fit for the worst-case lot (Lot 1) is generated using the pooled slope for the three lots as illustrated in Figure 12.4. The 90% confidence interval for that line fit is used in order to derive a lower one-sided 95% confidence bound on the fit line. The analysis reports the earliest intersection of the lower 95% confidence bound for Lot 1

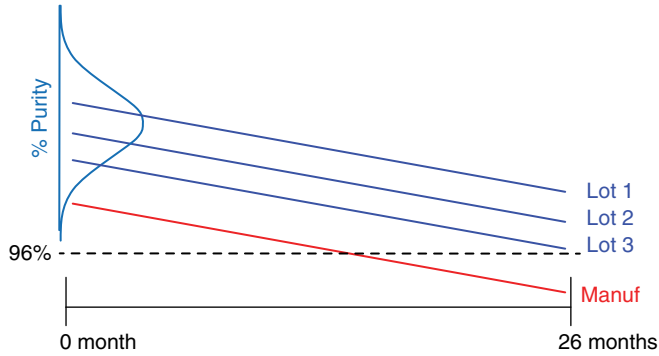


Figure 12.5 Purity monitored over 26 months (assigned shelf life) for a manufactured lot with a release purity outside of the distribution of the three stability lots used to assign the shelf life.

with the lower limit of the purity specification at 26 months. This then becomes the shelf-life specification for purity for the hypothetical drug product.

The shelf life determined on the basis of the analysis of purity versus time data in this way may be used to predict that the three stability study lots will maintain 96% or greater purity through 26 months, with 95% confidence. This statistically derived result does not, however, ensure that future drug product lots that are released with initial purity results outside the distribution for these three stability lots will maintain the required purity. This is illustrated schematically in Figure 12.5, where a commercial lot with the same slope, but with initial purity outside the distribution of the three stability lots, does not meet the 26-month shelf-life requirement.

12.7.1.3 Use of Stability Data to Establish Release Specification

The latter possibility may be mitigated by using the stability data to calculate the corresponding lot release specification. This enables the lot release specification to be set to ensure (with 95% confidence) that released drug product will maintain purity within specification throughout the intended shelf life. This is illustrated in Figure 12.6 for an intended shelf life of 24 months (time required to release, store, and distribute material). This again assumes that the degradation slopes for the additional lots are equivalent to the pooled slope for the three stability lots used to assign the shelf life.

The purity release specification in this case may be determined using values obtained from the statistical analysis of the stability lots used to determine shelf life. Assuming an intended shelf life that ensures adequate time to distribute the drug product (24 months), the equation required to calculate the desired release specification for purity is

$$\text{Release limit} = 96\% - 24 \cdot b + t_{0.95,\text{df}} \sqrt{\left(24 \cdot s_{\text{b}}\right)^2 + s_{\text{Assay}}^2}$$

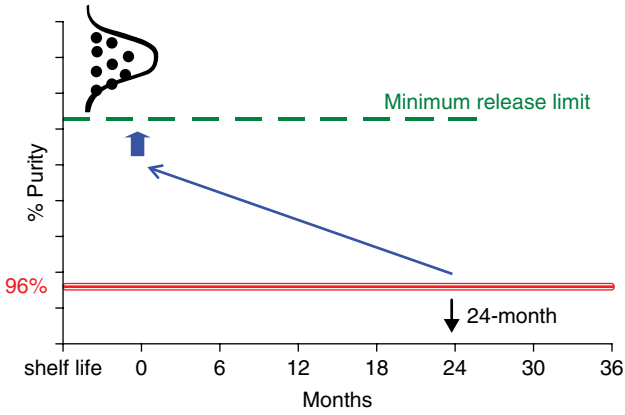


Figure 12.6 Minimum purity release specification to assure meeting a shelf-life requirement of 24 months. Arrows represent the estimated loss in purity by 24 months on stability and the combined slope and release assay variabilities.

Table 12.7 Summary of analysis of individual lots with root mean square error (s_{Assay}).

Summary of analysis	
RSquare	0.9570
RSquare adj	0.9450
Root mean square error (s_{Assay})	***0.2543***
Mean of response	97.6994
Observations	24

where b is the pooled slope from the purity versus time (stability) plots, $t_{0.95}$ and df are values derived from statistical tables of the t-distribution that ensures 95% confidence, s_b is the standard error of the pooled slope, and s_{Assay} is the release purity assay variability. These values for the previous purity analyses can be found in Tables 12.7 and 12.8.

Using estimates of the assay variability ($s_{Assay} = 0.2543$), the slope ($b = -0.0863\%$/month), the standard error of the slope ($s_b = 0.0047$), and a t-value from statistical tables with 18 degrees of freedom ($t_{95,18} = 1.734$) together with intended shelf life (24 months), the release limit for purity to support 96% purity at the end of the 24-month shelf life is calculated as

$$\text{Release limit} = 96\% - 24 \cdot (-0.0863) + 1.734\sqrt{(24 \cdot 0.0047)^2 + 0.2543^2} = 98.6\%.$$

Table 12.8 Pooled slope estimate (*b*) and standard error of the slope (s_b).

Term	*b* (%/month)	s_b	*t*	*P*(>*t*)
y-Intercept	98.8650	0.0828	1193.88	<0.0001
Lot 1	−0.4210	0.0749	−5.62	<0.0001
Lot 2	0.4631	0.0749	6.18	<0.0001
Lot 3	−0.0420	0.0749	−0.56	0.5807
Time	***−0.0863***	***0.0047***	−18.31	<0.0001

12.8 Summary

ICH Q5C provides guidance on the core stability package required for the registration of biological entities, in analogy with the parent guideline ICH Q1A(R2) that addresses the same topic focusing on small molecule therapeutics. There are many similarities between what is required to be performed for biologics and for small molecule therapeutics; however the nature of biological substances often necessitates more complex and different approaches. Biologicals are often complex macromolecules where maintenance of molecular conformation (high-order structure) is crucial in maintaining biological activity. As such, biologicals are more labile and may be particularly sensitive to environmental factors during storage, and stringent storage conditions may be necessary to avoid degradation and to maintain activity. Complex analytical methodologies may also be required to measure degradation and to assay biological activity. ICH Q5C articulates the regulatory expectations regarding the stability testing package that should be provided in support of marketing applications and should be considered in conjunction with, not independently of, ICH Q1A(R2).

References

1 ICH Q5C: Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products. 1996.
2 ICH Q1A(R2): Stability Testing of New Drug Substances and Products. 2003.
3 Manning MC, Chou DK, Murphy BM, Payne RW, Katayama DS. Stability of protein pharmaceuticals: an update. *Pharm Res*, 2010. 27(4): 544–575.
4 ICH Q5E: Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process. 2004.
5 Kumru OS, Joshi SB, Smith DE, Middaugh CR, Prusik T, Volkin DB. Vaccine instability in the cold chain: mechanisms, analysis and formulation strategies. *Biologicals*, 2014. 42(5): 237–259.

6 Moussa EM, Panchal JP, Moorthy BS, Blum JS, Joubert MK, Narhi LO, Topp EM. Immunogenicity of therapeutic protein aggregates. *J Pharm Sci*, 2016. 105(2): 417–430.

7 Narhi LO, Schmit J, Bechtold-Peters K, Sharma D. Classification of protein aggregates. *J Pharm Sci*, 2012. 101(2): 493–498.

8 Singh SK, Afonina N, Awwad M, Bechtold-Peters K, Blue JT, Chou D, Cromwell M, Krause H-J, Mahler H-C, Meyer BK, Narhi L, Nesta DP, Spitznagel T. An industry perspective on the monitoring of subvisible particles as a quality attribute for protein therapeutics. *J Pharm Sci*, 2010. 99(8): 3302–3321.

9 Roberts CJ. Protein aggregation and its impact on product quality. *Curr Opin Biotechnol*, 2014. 30: 211–217.

10 Vlasak J, Ionescu R. Fragmentation of monoclonal antibodies. *MAbs*, 2011. 3(3): 253–263.

11 Piszkiewicz D, Landon M, Smith EL. Anomalous cleavage of aspartyl-proline peptide bonds during amino acid sequence determinations. *Biochem Biophys Res Commun*, 1970. 40(5): 1173–1178.

12 Bee JS, Tie L, Johnson D, Dimitrova MN, Jusino KC, Afdahl CD. Trace levels of the CHO host cell protease cathepsin D caused particle formation in a monoclonal antibody product. *Biotechnol Prog*, 2015. 31(5): 1360–1369.

13 Robert F, Bierau H, Rossi M, Agugiaro D, Soranzo T, Broly H, Mitchell-Logean C. Degradation of an Fc-fusion recombinant protein by host cell proteases: identification of a CHO cathepsin D protease. *Biotechnol Bioeng*, 2009. 104(6): 1132–1141.

14 Chirino AJ, Mire-Sluis A. Characterizing biological products and assessing comparability following manufacturing changes. *Nat Biotechnol*, 22: 1383–1391.

15 Liu H, Gaza-Bulseco G, Sun J. Characterization of the stability of a fully human monoclonal IgG after prolonged incubation at elevated temperature. *J Chromatogr B Analyt Technol Biomed Life Sci*, 2006. 837(1–2): 35–43.

16 Salinas BA, Sathish HA, Shau AU, Carpenter JF, Randolph TW. Buffer-dependent fragmentation of a humanized full-length monoclonal antibody. *J Pharm Sci*, 2010. 99(7): 2962–2974.

17 Yan B, Yates Z, Balland A, Kleemann GR. Human IgG1 hinge fragmentation as the result of H_2O_2-mediated radical cleavage. *J Biol Chem*, 2009. 284(51): 35390–35402.

18 Gaza-Bulseco G, Faldu S, Hurkmans K, Chumsae C, Liu H. Effect of methionine oxidation of a recombinant monoclonal antibody on the binding affinity to protein A and protein G. *J Chromatogr B Analyt Technol Biomed Life Sci*, 2008. 870(1): 55–62.

19 Pan H, Chen K, Chu L, Kinderman F, Apostol I, Huang G. Methionine oxidation in human IgG2 Fc decreases binding affinities to protein A and FcRn. *Protein Sci*, 2009. 18(2): 424–433.

20 Mo J, Yan Q, So CK, Soden T, Lewis MJ, Hu P. Understanding the impact of methionine oxidation on the biological functions of IgG1 antibodies using hydrogen/deuterium exchange mass spectrometry. *Anal Chem*, 2016. 88(19): 9495–9502.

21 Qi P, Volkin DB, Zhao H, Nedved ML, Hughes R, Bass R, Yi SC, Panek ME, Wang D, Dalmonte P, Bond MD. Characterization of the photodegradation of a human IgG1 monoclonal antibody formulated as a high-concentration liquid dosage form. *J Pharm Sci*, 2009. 98(9): 3117–3130.

22 Li Y, Polozova A, Gruia F, Feng J. Characterization of the degradation products of a color-changed monoclonal antibody: tryptophan-derived chromophores. *Anal Chem*, 2014. 86(14): 6850–6857.

23 Pavon JA, Li X, Chico S, Kishnani U, Soundararajan S, Cheung J, Li H, Richardson D, Shameem M, Yank X. Analysis of monoclonal antibody oxidation by simple mixed mode chromatography. *J Chromatogr A*, 2016. 1431: 154–165.

24 Zhou S, Zhang B, Sturm E, Teagarden DL, Schöneich C, Kolhe P, Lewis LM, Muralidhara BK, Singh SK. Comparative evaluation of disodium edetate and diethylenetriaminepentaacetic acid as iron chelators to prevent metal-catalyzed destabilization of a therapeutic monoclonal antibody. *J Pharm Sci*, 2010. 99(10): 4239–4250.

25 Geiger T, Clarke S. Deamidation, isomerization, and racemization at asparaginyl and aspartyl residues in peptides. Succinimide-linked reactions that contribute to protein degradation. *J Biol Chem*, 1987. 262(2): 785–794.

26 Rivers J, McDonald L, Edwards IJ, Beynon RJ. Asparagine deamidation and the role of higher order protein structure. *J Proteome Res*, 2008. 7(3): 921–927.

27 Licensed under CC BY-SA 3.0 via Wikimedia Commons—http://commons.wikimedia.org/wiki/File:Deamidation_Asn_Gly.png#mediaviewer/File:Deamidation_Asn_Gly.png. No machine-readable author provided. "http://commons.wikimedia.org/wiki/User:WillowW" WillowW assumed (based on copyright claims). (accessed April 9, 2017).

28 Yan B, Steen S, Hambly D, Valliere-Douglass J, Vanden Bos T, Smallwood S, Yates Z, Arroll T, Han Y, Gadgil H, Latypov RF, Wallace A, Lim A, Kleemann GR, Wang W, Balland A. Succinimide formation at Asn 55 in the complementarity determining region of a recombinant monoclonal antibody IgG1 heavy chain. *J Pharm Sci*, 2009. 98(10): 3509–3521.

29 Liu YD, van Enk JZ, Flynn GC. Human antibody Fc deamidation *in vivo*. *Biologicals*, 2009. 37(5): 313–322.

30 Dillon TM, Ricci MS, Vezina C, Flynn GC, Liu YD, Rehder DS, Plant M, Henkle B, Li Y, Deechongkit S, Varnum B, Wypych J, Balland A, Bondarenko PV. Structural and functional characterization of disulfide isoforms of the human IgG2 subclass. *J Biol Chem*, 2008. 283(23): 16206–16215.

31 Liu H, May K. Disulfide bond structures of IgG molecules: structural variations, chemical modifications and possible impacts to stability and biological function. *MAbs*, 2012. 4(1): 17–23.

32 Franey H, Brych SR, Kolvenbach CG, Rajan RS. Increased aggregation propensity of IgG2 subclass over IgG1: role of conformational changes and covalent character in isolated aggregates. *Protein Sci*, 2010. 19(9): 1601–1615.

33 Bozhinov AS, Boyanova M, Niwa T, Ivanov I, Mironova R. Evidence for the presence of glycation adducts in protein therapeutics. *Biotechnol Biotechnol Equip*, 2010. 24(2): 1904–1909.

34 Miller AK, Hambly DM, Kerwin BA, Treuheit MJ, Gadgil HS. Characterization of site-specific glycation during process development of a human therapeutic monoclonal antibody. *J Pharm Sci*, 2011. 100(7): 2543–2550.

35 Fitzpatrick K, Kendrick B, Santos C, Green P, Zhang B, Hunt D, Ronk M, Luo Y. Freeze-dry mediated formation of 5-(hydroxylmethyl)furfural, in *Developments in Biotechnology and Bioprocessing*. 2013, American Chemical Society, pp 129–145.

36 FDA: Guidance for industry: Container and Closure System Integrity Testing in Lieu of Sterility Testing as a Component of the Stability Protocol for Sterile Products. 2008.

37 European Medicines Agency: Quality of Medicines Questions and Answers: Part 2 http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/q_and_a/q_and_a_detail_000072.jsp#section16, 2009 (accessed April 9, 2017).

38 Corvari V, Narhi LO, Spitznagel TM, Afonina N, Cao S, Cash P, Cecchini I, DeFelippis MR, Garidel P, Herre A, Koulov AV, Lubiniecki T, Mahler HC, Mangiagalli P, Nesta D, Perez-Ramirez B, Polozova A, Rossi M, Schmidt R, Simler R, Singh S, Weiskopf A, Wuchner K. Subvisible (2-100 μm) particle analysis during biotherapeutic drug product development: part 2, experience with the application of subvisible particle analysis. *Biologicals*, 2015. 43(6): 457–473.

39 Narhi LO, Corvari V, Ripple DC, Afonina N, Cecchini I, Defelippis MR, Garidel P, Herre A, Koulov AV, Lubiniecki T, Mahler HC, Mangiagalli P, Nesta D, Perez-Ramirez B, Polozova A, Rossi M, Schmidt R, Simler R, Singh S, Spitznagel TM, Weiskopf A, Wuchner K. Subvisible (2-100 μm) particle analysis during biotherapeutic drug product development: part 1, considerations and strategy. *J Pharm Sci*, 2015. 104(6): 1899–1908.

40 ICH Q1B: Stability Testing: Photostability Testing of New Drug Substances and Products. 1996.

41 ICH Q1D: Bracketing and Matrixing Designs for Stability Testing of New Drug Substances and Products. 2002.

42 EMA/CHMP/BWP/534898/2008: Guideline on the requirements for quality documentation concerning biological investigational medicinal products in clinical trials. 2010.

43 ICH Q1E: Evaluation for Stability Data. 2003.

13

Q5D Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products

Mark Plavsic

Lysogene, Cambridge, MA, USA

13.1 Introduction

Cell substrates are cells used to manufacture a biological product. For recombinant protein products, the cell substrate constitutes engineered cells carrying the gene encoding the therapeutic protein. The manufacture of mammalian cell-derived products (recombinant proteins, monoclonal antibodies (mAb), viral vaccines, viral vectors used in gene therapy, etc.) cannot be realized without the use of well-defined and properly characterized cell substrates. These cell substrates are often referred to as the production starting material, and they are viewed as some of the most important assets a biotechnology company may possess. The construction, selection, production, testing, characterization, and storage of cell substrates therefore represent some of the most fundamental and vital prerequisites for ensuring successful manufacture of high quality biological products having the requisite safety, purity, and potency. The International Conference on Harmonisation (ICH) guideline Q5D [1] is intended to provide guidance to biologics manufacturers on the regulatory expectations around the derivation, generation, and characterization of the production cell substrate. Considered in scope for this guidance are all production cell substrates subject to a cell banking system.

The purpose of this chapter is to examine the practical implementation of the Q5D guideline using Chinese hamster ovary (CHO) cells as a model. The principles outlined may be broadly applicable to other mammalian (metazoan), and to a large extent protozoan, host cell systems used in recombinant protein production. We, however, recognize that protozoan cell substrates may have certain specifics that are not common to mammalian cells and

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hence require additional considerations outside the scope of this chapter; nevertheless the principles outlined here should have broad applicability across a range of cell types.

13.2 Source, History, and Generation of the Cell Substrate

The initial portion of the Q5D guidance outlines the expectations around the supportive documentation expected for the overall history of the production cell substrate. As indicated in the guidance, extensive deficiencies in the documented history of the cell line will result in increased reliance (e.g., scrutiny) on other aspects of cell line characterization.

13.2.1 Generation of the Production Cell Line and Pre-Master Cell Bank

Creation of a production cell line consists of several steps, which may include:

- Selection of a parental host cell line
- Construction of high expression vectors
- Transfection of the vectors into the parental cell
- Selection of stable and high producing cell pools
- Cloning of the high producing stable cell pools
- Clone evaluation and characterization
- Clone banking (pre-master cell bank (MCB))

13.2.2 Host Cells and Parental Cell Line

Historically, generation of a cell substrate typically begins with the selection of an untransfected host cell, also known as the parental cell line. In the case of a CHO-based manufacturing platform, the host cell/parental cell line can be, but is not limited to, CHO-DG44, CHO DXB11, CHO-K1SV, CHO-S, or a cell line derived from one of these. Careful management of the parental cell line is essential, as it serves as the platform for creation of future transfected cell lines (production cells, cell substrates) to be used in new product development and manufacture. Since the original host cells may be genetically malleable, exposure to certain growth conditions followed by cell cloning may be carried out at the host cell level in order to select a cell clone with specific growth and metabolic traits. At the same time, adaptation to animal origin-free or chemically defined conditions using a "platform" basal medium is recommended. In this context, the basal medium is defined as a formulation platform that may be used to culture multiple cell lines/clones from early clone selection to medicinal product manufacture without the need for basal medium change (medium replenishment regimens may need to be optimized, however, for different cell lines expressing different molecules). The host cells with desired traits, derived

after cloning and adaptation to a platform cell culture medium, are banked, tested, and maintained for future needs. The host cells should have a clear source, history, culture requirements, and culture properties and should be tested for the presence of adventitious agents. Clear and complete records must be in place defining the source, traceability, genealogy, history, identity, purity, and quality of the host cells.

13.2.3 Cell Engineering and Protein Expression Technologies

The quantity of recombinant protein expressed by a cell culture is dependent on the time integral of viable cell density (IVCD) and specific protein productivity of the cells. To improve IVCD, cell line engineering strategies focus on extending the longevity of cell culture, accelerating the specific growth rate, and increasing the maximum viable cell density. Specific cell productivity can be influenced by careful vector design, expression platform, and cell line engineering [2].

A variety of cell line engineering strategies are used that target diverse cellular functions of CHO cells, such as apoptosis, autophagy, proliferation, regulation of cell cycle, protein folding, protein secretion and metabolite production, and IVCD.

The specific productivity (SPR) of a cell culture can be increased through the modulation of transcriptional activity. This is typically done through expression vector engineering modulating the co-expression of product and selection marker genes, the stringency of the selection markers, the DNA regulatory elements carried on the vector, and the targeting of the integration site on the host cell genome. Different protein expression technologies are available that rely on transfection vector and expression cassette design and vector integration. Examples include:

- Internal ribosome entry site (IRES)
- Selection marker attenuation
- Matrix attachment regions (MARs)
- Site-specific recombination (e.g., Cre-LoxP recombinase, lambda integrase)
- Artificial chromosome expression

Specific genome integration of the transgene is further boosted by the discovery of genome editing tools such as zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALENs), meganucleases, and clustered regularly interspaced short palindromic repeats-associated endonucleases (CRISPR-Cas). These tools offer an opportunity to cut (edit) host cell DNA molecules at certain known locations where a transgene can be inserted. The resulting double-stranded DNA break at the targeted gene locus is subsequently repaired by the co-transfection of a transgene with a homologous region to the cut site. This results in the transgene integration at the nuclease-targeted cleavage site via homologous end joining [2, 3].

Currently, cell line development technologies are based on either the methotrexate (MTX) amplification technology that originated in the 1980s or the glutamine synthetase (GS) system [4, 5]. Both systems make use of a specific drug to inhibit a selectable enzyme marker essential for cellular metabolism. MTX inhibits the enzyme dihydrofolate reductase (DHFR) in the MTX amplification system, and methionine sulfoximine (MSX) inhibits the enzyme GS in the GS system. Complementary to these drug/enzyme pairs are cell lines that are deficient in these enzymes. While CHO cell lines deficient in DHFR have been established since the 1980s, those deficient for GS have been developed only recently. After transfection with vectors containing the expression cassettes for the recombinant protein and selection marker genes, the cells are selected for stable transfectants and gene-amplified with the selection drug, for example, MTX or MSX. With the MTX system, gene amplification can be performed by applying increasing levels of selection drug. In this case, gene amplification refers to the increase in recombinant gene copy number in the cells commonly associated with the applications of MTX. MTX concentration can also be increased stepwise to further increase cell protein productivity by further gene amplification.

13.2.4 Transfection

In the production cell line development context, transfection is a procedure that introduces foreign genes of interest (g.o.i.) (transgenes) into living cells to generate a production cell line capable of efficiently expressing the protein of interest. The transfected genetic materials exist in cells either stably or transiently, depending on a number of factors. For stable transfection, the introduced g.o.i. (transgene) is integrated into the host cell genome, leading to sustained transgene expression even after the host cells replicate. In contrast, transiently transfected g.o.i. are not integrated in the host cell genome and the protein is therefore expressed for a limited duration. Transiently expressed genes may be diluted and lost after subsequent cell divisions.

There are many transfection methods to choose from, and these can be classified into biological, chemical, and physical methods:

Biological methods rely on the use of a virus to mediate transfection. Typically, a retrovirus (e.g., murine leukemia virus) is used to achieve a sustainable transgene expression due to the fact that retroviruses integrate into the host cell genome. Other viruses used in biological transfection (transduction) are herpes simplex, adenovirus, adeno-associated virus, vaccinia virus, and Sindbis virus. Virus-mediated transfection is usually of high efficiency; however, viruses have limited space for packaging large transgenes. In addition, viruses can be cytotoxic to the cells and may also disrupt normal cell physiology. Retroviruses integrate randomly in the host genome and may disrupt tumor suppression genes, activate oncogenes, or interrupt the functions of normal host cell genes [6].

Chemical transfection methods are the oldest and most widely used in contemporary research and development. The chemicals commonly used include cationic polymers (DEAE-dextran, polybrene, polyethylenimine), calcium phosphate, cationic lipids (lipofectin, lipofectamine, DOTAP, DOTMA), and cationic amino acids. The chemical methods display relatively good transfection efficiency, low cytotoxicity, no mutagenesis, and no size limitation of the packaged nucleic acid/g.o.i [6, 7].

Physical transfection methods rely on the use of certain physical tools to deliver a g.o.i. into the cell. The methods include electroporation, microinjection, gold particle delivery (biolistic), and laser-based transfection. Electroporation is the most widely used physical tool because it is straightforward and reliable, easy, and rapid, and it can transfect large number of cells in a short time once the transfection conditions are optimised [6, 7].

There are many factors that can influence transfection efficiency. Some are specific to the target cell, while others are method specific. Cell-related factors affecting transfection include cell density, cell size, replication state, passage number, health of cells, quality of cell culture medium, and concentration of the transgene. Method-specific factors include quality of reagents, experimental conditions, concentration, pH, voltage, capacitance, etc.

13.2.5 Selection of Stable High Producing Cell Pools

After transfection of the host cell line with the expression vector containing the g.o.i. and selection marker(s), the transfected cells are grown in selection medium (i.e., medium containing the selection agents to allow proliferation of stable pools). When gene amplification systems are used, concentrations of the selection drug (e.g., MTX) can be increased stepwise to derive cell clones that are more productive. Pools are screened for expression of the protein of interest, and the best producing pools are isolated and cloned for individual cell clone selection.

A research cell bank may be generated for each pool as a backup strategy to mitigate business risk associated with the development process.

13.2.6 Post-Transfection Analysis of Cells

After any transfection process, it is important to assess the efficiency of the transfection and the impact, if any, on the cells. Analysis of transfection can be as simple as confirming the expression of the g.o.i. In many cases, however, assessment involves determining the total expression level of the g.o.i., the number of transfected cells within the population, and visual confirmation of the expressed protein of interest. The analytical tools used in this context include the following [8, 9]:

- Flow cytometry, to determine the number of positive cells within a transfected population
- Fluorometry, to quantify the expression level of the protein of interest based on fluorescence

- Luminometry, for quantitative analysis of protein expression based on expression of luciferase
- Microscopy, to directly visualize transfected cells
- Real-time quantitative PCR (RT-qPCR), to quantify the expression level of the transgene
- Spectrophotometry, to determine transfection efficiency by measuring colorimetric changes
- Western blot analysis, to identify and quantify total expression of the transgene from a population of cells

Analytical tool selection is based on a number of factors and is specific application dependent. In most cases, several analytical tools are used for more thorough clone/pool evaluation.

13.2.7 Cloning and Selection of High Producing Stable Clones

Cloning is the process of isolating the single best producing stably transfected cells (monoclones) from the initial best producing cell pool(s). In addition to being a regulatory requirement, there are several reasons why it is important to ensure monoclonality [2].

As a result of the random integration of foreign g.o.i. and subsequent disruption of the genome by drug selection gene amplification systems, the cell pools from which clones are obtained during cell line development may be highly heterogeneous, resulting in a wide range of cell SPR and a low percentage of highly producing cells. Hence, in situations where high recombinant protein productivity is essential for product commercial viability (e.g., the production of mAb therapeutics), thousands of clones may have to be screened to obtain a set of production clones. While the screening process can be assisted with modern technologies and robotics, the production cell line development process is still a time-, labor-, and capital-intensive endeavor that typically requires 6–12 months to complete.

The most widely used method of cloning involves two rounds of limiting dilution cloning (LCD) into 96- or 360-well plates at a dilution level that is sufficient to statistically predict monoclonality. Recently some regulators have recommended more robust visual documentation of clonality (see Figure 13.1) since the LCD method cannot rule out doublets of "sticky" cells. Out of several hundreds or even thousands of screened clones, typically 20–30 from each pool are selected and expanded in shake flasks. After additional evaluation of growth, productivity, and some product quality attributes, a subset (e.g., 6–10) of clones is selected for cell line stability determination, expanded product quality testing, and initial process assessment. Based on these data, a lead clone and a backup clone(s) are chosen. A second round of cloning may be performed on the best producer in order to provide better monoclonality; alternatively, one round of cloning followed by the imaging of well plates on day 0 of plating

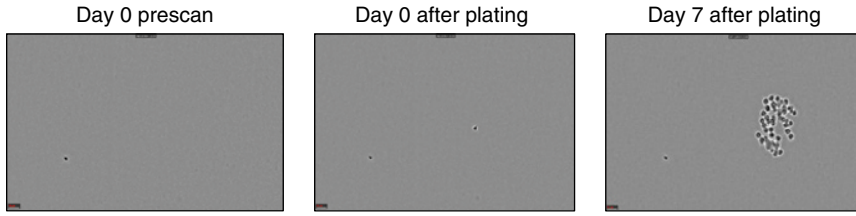


Figure 13.1 Demonstration of monoclonality using a well plate imaging technology.

The photographs were taken on day 0 before and after plating of cells. Cells were incubated to allow clones to develop. Only images from the wells that appear to contain a single cell clone were examined for day 0 to confirm monoclonality:

Day 0 prescan: This is a picture prior to plating cells; the plate contains only cell culture medium. There is a defect in the well that looks similar to a cell but is an artifact because it is there prior to plating and never changes. *Day 0 after plating*: This image provides the needed evidence that only a single cell was deposited. *Day 7 after plating*: Expanded cells from a single clone. All images shown are of the area of the well with the single cell clone outgrowth. Full well image is used to demonstrate that no other cell or outgrowth exists in the rest of the well (not shown).

and subsequent days can be employed (Figure 13.1). Selecting a clone with SPR of >50 pg/cell/day is possible through this process. The genetic stability of the clone and gene sequencing of the transgene do not constitute clonality, although these may be used among other analytical tools to characterize the selected clone.

Importantly, additional steps of cultivating the cells and protein titer analysis (enzyme-linked immunosorbent assay (ELISA) or Octet technology) are used to determine the protein productivity of the clones.

Advancements in clone screening technologies can reduce the time and effort required to identify and select the rare high producing cell clones. Several such technologies are briefly discussed in this section [2].

13.2.7.1 Fluorescence-Activated Cell Sorting (FACS)-Based Screening

Fluorescence-activated cell sorting (FACS) sorters can simultaneously monitor, at a high rate (10^8/h), the levels of multiple fluorescence wavelengths associated with a cell. Cells to be analyzed enter the FACS sorter singularly as a moving focused stream, and they are interrogated by one or more laser beams. The resulting fluorescence from the cell is measured by relevant optical detectors, and the collected data are quantified and analyzed. The instrument then applies a charge to the droplet containing the cell to sort it into specific collection tubes or well plates. Depending on the fluorescence signal, cell parameters such as granularity and cell size can also be obtained. However, accuracy of the FACS-based screening of high producer cell clones is dependent on the fluorescence signal that remains associated with the cell. Hence, it is more suited

for selection of high producing cell clones that do not secrete the recombinant protein into the culture medium or that have a cell surface reporter protein co-expressed with the recombinant protein of interest [2, 10, 11].

13.2.7.2 ClonePix

The ClonePix system is an automated colony picker that is capable of screening large numbers of clones and identifying high producing clones within a short period of time [12]. The process of ClonePix is initiated by cultivating single cells in a semisolid medium to allow the formation of individual colonies. The high viscosity of the medium allows the progeny of the single cell to remain as a single colony and also traps the secreted recombinant proteins in close proximity to the secreting colonies. These secreted recombinant proteins are captured by fluorescein isothiocyanate (FITC)-conjugated antibodies that were previously added to the semisolid medium. Upon capturing of the expressed recombinant proteins by the antibodies, these are deposited as immunoprecipitates around the secreting cell clone and hence form a halo fluorescence structure. Consequently, clones are ranked according to the fluorescence intensity of the halo structure. High-ranking high producer cell clones are then aspirated by micro-pins and transferred to a new well plate for further characterization. The entire process of imaging 10,000 cell clones and selection of high producer cell clones are completed within an hour, and it is sensitive enough to isolate rare high producing clones that represent only 0.003% of the cell population. Due to the high-throughput nature of this method, the ClonePix system has been used in different studies to consistently select high producer cell clones expressing a range of recombinant proteins and antibodies [2].

As in the case of the manual cloning methods, to ensure monoclonality of the production cell line, at least two rounds of cloning using the FACS and ClonePix technologies should be performed:

13.2.7.3 Cell Xpress™

Cell Xpress™ technology consists of the Cell Xpress™ software module and the Laser-Enabled Analysis and Processing (LEAP™) platform that are based on the principle of laser-mediated semiconductor manufacturing technologies. An advantage of the LEAP™ platform is that it is designed for high-throughput operation, and hence the entire screening process is fully automated and accomplished by robotics. The Cell Xpress™ technology combines live cell imaging and laser-mediated cell manipulation to identify and purify the highest producer cell clone in a sample well. Multicolor live cell imaging of the Cell Xpress™ technology is achieved through the simultaneous use of fluorescence detection reagents that associate specifically with either

the cell clones or the expressed recombinant proteins. Furthermore, the sample wells are coated with a capture matrix to mediate in situ capture of expressed recombinant proteins, as most of the recombinant proteins are secreted out of the cell. For example, if therapeutic antibodies are expressed, protein G is commonly used to capture the secreted antibodies. Through measurement of the fluorescence intensity of the respective detection reagents, each cell in the same well will be ranked based on the amount of secreted recombinant protein. Hence, the highest ranking cell in a well will be identified as the most suitable high producer cell clone. Subsequently, laser-mediated cell purification will commence whereby the laser beam is directed to the lower-ranking cell clones in the same well via large field-of-view optics and galvanometer steering to induce photomechanical lysis. Any high producing cell that remains in the well will be allowed to grow and will be transferred to a larger well for expansion [2].

13.2.8 Clone Evaluation and Banking

When a large number of high producing clones are isolated, clone expansion in shake flasks will enable initial evaluation of selected clones. Cells are evaluated for their growth characteristics, media utilization, metabolic production, doubling time, size, morphology, viability, apoptosis, and protein expression. Transgene sequencing and cell line stability can also be evaluated at this stage. Cell growth, productivity, product quality, and genetic stability demonstrated for a defined number of generations are required for commercial manufacturing. Finally, if not attempted earlier, clone adaptation into a new chemically defined or animal origin-free cell culture medium can also be attempted at this stage. The result of this evaluation is triage and selection of clones with the most desired characteristics under the given culture conditions.

A pre-MCB is typically generated from an early passage research bank vial. It serves as the source bank for production cell bank generation. The pre-master cells are first tested for purity to ensure freedom from adventitious agents (bacteria, viruses, and mycoplasma) before a vial from this bank is transferred into the GMP facility.

13.2.9 Generation of GMP Cell Banks

It is a regulatory expectation that a continuous cell line used to produce a biological will be banked. The Q5D guidance therefore discusses at length the two-tiered GMP cell banking system and the principles underlying the characterization and testing of the cell banks. The GMP cell banks include an MCB and one or more working cell banks (WCB). These are manufactured in accordance with GMP principles, tested, and stored (cryopreserved) until further use in manufacturing.

13.2.10 Points to Consider in Cell Substrate Development

Development of production cell substrates, from the initial vector design and host cell selection through the generation of the GMP cell banks, is very labor intensive. These activities require specialized technical expertise, equipment, and a wide range of reagents and raw materials. In order to ensure manufacture of a consistently high quality product and to meet current regulatory standards, it is critical to perform high quality and well-documented work throughout the whole process. Special attention should be paid to minimizing the risk from adventitious agent introduction, especially viruses and mycoplasma. These agents could persist in the cells as silent contaminants, often times hard to detect using standard testing technologies. Adventitious agents may be introduced via contaminated raw materials and reagents or from operators or the laboratory/manufacturing environment. It is therefore essential to introduce effective controls, such as selection of high quality and safe raw materials and reagents, use of good laboratory practices (GLP), aseptic laboratory techniques, properly cleaned and sanitized equipment and work areas, and properly trained operators.

All raw materials and reagents should be sourced from reputable suppliers with accompanying certificates and records. Avoidance of animal- and human-origin materials should be considered from the very earliest stages of cell line development and cell adaptation. Traceability and lineage of the cell substrates are essential and should be documented properly. All documentation associated with the expression vector design, host cell, transfection, cloning, analytical evaluation, cell banking, and raw materials used throughout the process should be well defined, detailed, and easy to retrieve, since each cell substrate will require regulatory approval.

Finally, a risk assessment should be performed at certain points (e.g., pre-MCB) to assess the risk of cross-contamination and to determine the level of biosafety testing specific for each cell substrate before GMP bank generation (see also Chapter 14).

13.3 Cell Bank Characterization

Cell line characterization is viewed as a key component of the assurance of product quality and safety, and this topic is discussed in detail in Q5D.

Characterization of cell banks occurs at several points, namely, the parental cells, pre-MCB, MCB, WCB, and the end-of-production cell (EOPC). The type and extent of characterization testing depends on the type of cell bank and the animal species of origin. Typically, the GMP-grade cell banks (i.e., MCB, WCB, EOPC) must be tested for identity, purity (cell purity and freedom from adventitious contaminants), karyology, tumorigenicity/oncogenicity, and genetic stability.

13.3.1 Identity Testing

Appropriate tests are performed to determine that the banked cell is what it is represented to be. Either phenotypic or genotypic characteristics may be used in identity testing. Tests of identity are generally performed on the MCB. In addition, limited identity testing is generally performed on each WCB. In the past, isoenzyme analysis has been sufficient to confirm the species of origin for cell lines derived from human or animal sources. More recent test methods such as mitochondrial cytochrome oxidase I (CO1) barcoding (animal cells) and short tandem repeat (STR) profiling (human cells) are now replacing isoenzyme analysis for this purpose [13]. The latter fulfill the regulatory recommendation that "state-of-the-art methods and technological improvements in cell substrate characterisation and testing" be used as they become available, "as long as the specificity, sensitivity, and precision of the newer methods are at least equivalent to those of existing methods" [1]. Demonstration of specific cellular markers, unique markers, expression of the desired product, cytogenetic analysis, DNA fingerprinting, and single nucleotide polymorphism (SNP) profiling are also part of the portfolio of identity testing methods.

13.3.2 Purity Testing

Purity of cell substrates is defined as absence of other types of cells and adventitious microbial agents such as bacteria, fungi, mycoplasma, and viruses. Cross-contamination of one cell line with another type of cells, although rare in the modern cell culture applications, could occur under certain circumstances [14]. Assays for cell line identity (authenticity) are capable of assuring freedom from other types of cells.

Cell culture systems are prone to contamination with adventitious microbial and viral agents. The risks of contamination are not theoretical; there have been a number of cases where virus contaminations have been detected during the production of biologics [15–23]. Contaminating agents may be associated with the cell substrates themselves (e.g., endogenous retroviruses (ERV)), or they may be inadvertently introduced during cell line development and cell culture manipulation through the use of contaminated reagents, raw materials, or a breach in GLP and GMP. CHO cells are known for their ability to generate endogenous, defective (i.e., noninfectious) retrovirus particles [24]. Contamination of CHO cell culture manufacturing processes with adventitious agents has also be documented. It has been demonstrated that CHO cells are susceptible/permissive to viruses from at least 11 viral families, including parvoviruses (e.g., mouse minute virus), reoviruses (e.g., reovirus and epizootic hemorrhagic disease virus), caliciviruses (e.g., vesivirus 2117), bunyaviruses (e.g., Cache Valley virus), and picornaviruses (e.g., encephalomyocarditis virus), to name a few [21].

The cell banks intended for use in GMP manufacture of biological products have to be shown to be free from contamination with virus, bacteria, fungi,

mycoplasma, and other microbial cell types. Very specific adventitious agent detection assays are used, and these are detailed in the ICH Q5A(R1) guidelines [25] (see also Chapter 10). Table 13.1 provides a general overview of adventitious agent testing using a CHO cell culture as a model. For more specific assay details, readers are referred to ICH Q5A(R1), WHO document TRS 878 Annex 3 [29], and the EMA guidance on virus safety evaluation of biotechnological investigational products [30]. Note that ICH Q5D does not specifically address testing requirements for parental cells and pre-MCB. The testing displayed for a pre-MCB in Table 13.1 is that which is recommended by the author.

13.3.3 Karyology, Tumorigenicity, and Oncogenicity

For drug products that are highly purified and that contain no cells (e.g., mAb), karyology and tumorigenicity testing are generally not considered necessary, provided that appropriate limits for residual host cell DNA are shown to be consistently met by either process validation studies or lot release testing. Karyology also is not required for non-diploid rodent cell lines. For products that are manufactured in genetically unmodified MRC-5 or WI-38 diploid cells, the cell substrates do not require karyology or tumorigenicity evaluation, since extensive characterization has already been performed and published for these cell lines. However, for each MRC-5 and WI-38 WCB generated, manufacturers should confirm, once, that the cells grown in the manner to be used in production are indeed diploid and have the expected lifespan. For new or previously uncharacterized diploid cell substrates, confirmation of diploid status by karyology or other ploidy analysis technologies should be presented, and tumorigenic potential should be established using cells from the MCB.

Tumorigenicity is defined as a property of the cell substrates to form tumors in an inoculated animal test system. These tumors arise from the inoculated cell substrate and are derived from the same cells as the cell substrate. Although in vitro assays are available, in vivo tests remain the standard for assessing tumorigenicity. Cells from the MCB or WCB, propagated to the proposed in vitro cell age used for production or beyond (i.e., EOPC), should be examined for tumorigenicity. The extra population doublings ensure that the results of the tumorigenicity test can be used in the assessment of overall safety of the product even under the assumption of a "worst-case" situation and therefore provides a safety buffer.

In vivo tumorigenicity assay. The test cell line and the control cells are each injected into separate groups of 10 athymic mice (Nu/Nu genotype) at 4–7 weeks of age. All animals are examined weekly by observation and palpation for a minimum of 4 months for evidence of tumor formation at the site of injection or other sites (metastasis), which indicates tumorigenicity of the cell substrate.

Table 13.1 Cell bank adventitious agent testing for a Chinese hamster ovary production cell.

Assay type	Assay purpose	Pre-MCB (suggested minimum testing)	MCB	WCB	Cells at limit of in vitro age
Microbial testing					
Sterility	Detection of bacterial and fungal agents	Yes	Yes	Yes	Yes
Mycoplasma	Detection of adventitious mycoplasma	Yes	Yes	Yes	Yes
Adventitious virus testing					
In vitro assay	Detection of adventitious viruses in cell culture systems	Yes	Yes	Yes	Yes
In vivo assay	Detection of adventitious viruses in several animal models	No	Yes	No	Yes
Retrovirus testing					
Electron microscopy	Visualization and quantitation of viruslike particles	Yes	Yes	No	Yes
Reverse transcriptase (RT)	Detection of retroviral RT enzyme	No	Yes	No	Yes
Infectivity	Detection of infectious retroviruses	No	Yes	No	Yes
Species-specific virus testing					
HAP	Detection of antibodies for specific viruses in hamsters after test article inoculation	No	Yes	No	No
MAP	Detection of antibodies for specific viruses in mice after test article inoculation	No	Yes	No	No
Porcine viruses [1]	Detection of porcine viruses	No	As appropriate	As appropriate	As appropriate
Bovine viruses [2]	Detection of bovine viruses	No	As appropriate	As appropriate	As appropriate

(*Continued*)

Table 13.1 (Continued)

Assay type	Assay purpose	Pre-MCB (suggested minimum testing)	MCB	WCB	Cells at limit of in vitro age
Detection of other viruses					
MVM (MMV)	Testing for these and additional viruses, although not specifically required by the ICH Q5A and ICH Q5D, should be decided based on a risk assessment that is specific for each cell substrate, considering the origin, source, animal-origin raw materials, and culture history of the cells (see also Chapter 14) Addition of testing for other species-specific agents should be considered on a case-by-case basis depending on the origin of the cells and manufacturing process employed. Utility of next-generation sequencing (NGS) for cell substrate testing and characterization should also be considered				
Vesivirus 2117					
LCM virus					
Bovine polyomavirus					
Porcine circovirus 1/2					
Bovine parvovirus 2/3					
Other specific agents					

Testing for porcine and bovine viruses is performed in cases where bovine- and porcine-derived components are used in the generation and production of cell substrates [1, 2]. Test methods are designed to comply with the 9 CFR [26] and EMA [27, 28] guidance documents for bovine- and porcine-derived materials. Since these methods have limitations and certain viruses may not be detected, additional approaches should be considered to ensure safety of cell banks. The current EMA guidance on bovine serum [27] used in manufacture of human medicinal products stipulates that "serum suppliers and users should also be aware of emerging bovine viruses and are encouraged to investigate the presence of such agents in bovine serum and to take appropriate action to eliminate or reduce the presence of any novel virus in serum."

13.3.3.1 In Vitro Tumorigenicity Assay

This assay relies on the ability of transformed cells to remain viable and form colonies when suspended in soft agarose due to a decreased requirement for cell–cell or cell–substrate adhesion for their proliferation. This property in soft agar is a useful technique for assessing the neoplastic potential of a cell substrate. In this assay, serial dilutions of the test cells and the control cells are inoculated into prepared agar-cell culture medium base. The cells are incubated for 4 weeks with regular microscopic observation for visible colonies of more than 10 cells, which indicate in vitro tumorigenicity of the cell substrate.

Oncogenicity is the property of an acellular agent to induce cells of an animal to become neoplastic cells. As such, tumors that arise in a tumorigenicity assay contain cells derived from the inoculated cells, while tumors that arise in an oncogenicity assay are derived from the host animal. Oncogenic activity from cell substrates could be due either to the cell substrate DNA (and perhaps other cellular components) or to an oncogenic viral agent present in the cells.

13.3.3.2 In Vivo Oncogenicity Test

Oncogenicity testing is performed on the MCB. Newborn (i.e., <3-day-old) nude mice, newborn hamsters, and newborn rats have been used to assess the oncogenic potential of cell lines. While the number of animals in a tumorigenicity test can be 10 per group, the number in an oncogenicity test should be larger due to the lower expected tumor incidence. All animals are examined weekly by observation and palpation for a minimum of 4 months for evidence of tumor formation at the site of injection or other sites (metastasis), which indicates the oncogenicity of the cell substrate.

Detailed description of methods for karyology and tumorigenicity analysis may be found in the WHO document "WHO Requirements for Use of Animal Cells as *in vitro* Substrates for the Production of Biologicals" [29].

13.3.3.3 Genetic Stability Testing

The concept of cell substrate stability is introduced in ICH Q5D and is further dealt with in ICH Q5B [31] (Chapter 11). Any form of genetic instability could potentially affect the quality of the final product, and it is therefore important to know if the cells in culture are changing in a way that could affect the nature or safety of the expressed product. The specific tests will vary according to the nature of the product, but the aim in general is to show consistency in the amount and characteristics of the transgene derived from cells within a few passages of the MCB or WCB with those derived from an EOPC. For recombinant protein products, emphasis will be on the protein sequence and posttranslational modifications. For cell lines containing DNA expression constructs, the stability of these constructs between the MCB/WCB and EOPC is normally determined. Several analytical methods are

available for stability testing. This topic is addressed in greater detail in ICH Q5B [31] (Chapter 11):

- The copy number of the construct and, if relevant, the sites of chromosomal insertion should be determined. This is accomplished by extending the sequencing into the transgene flanking regions.
- Fluorescent in situ hybridization may provide useful additional information.
- The sequencing of the construct within the cells is typically performed using either conventional or massively parallel sequencing (next-generation sequencing) technologies. With conventional sequencing, a consensus sequence is obtained, but with massively parallel sequencing, it is possible to determine the sequence of individual gene inserts or their transcripts.

Initial growth and productivity stability (beyond the limit of in vitro cell age for production use) are typically assessed using small-scale models early in the development process during clone selection. Growth and production stability are important for process economics and process robustness. However, from a regulatory viewpoint, consistent production refers to the ability to maintain the identity of the protein expressed by the production cell. To demonstrate suitability of the cell substrate for the manufacturing process, at least two time points should be evaluated: one using cells with a minimum number of population doublings and another using cells at or beyond the limit of in vitro cell age for production use. Biochemical analyses (peptide mapping, MALDI-TOF-MS, ESI-MS, SDS-PAGE, IEF, protein activity, or product quantification assays) and genetic techniques (sequencing, northern blotting, Southern blotting, etc.) are used to demonstrate construct integrity and to detect potential changes in copy number, mRNA level, and vector integration site with increasing cell age.

13.4 Summary

The guidance provided in ICH Q5D is essential for manufacturers intending to introduce a new molecule into clinical trials. Careful consideration of all of the requirements articulated within this guidance should be given prior to contemplating the creation of the production cells. It is much more difficult to satisfy the documentation requirements and to mitigate risk of adventitious agent contamination when focusing on these aspects only toward the end of the production cell generation process. Newer analytical technologies may be able to allay *concern* for cell lines with documentation deficiencies. For example, next-generation sequencing as applied to cell line purity testing has the capability of identifying unknown viral or mycoplasma contaminants that might be present in a cell bank due to exposure at some point to a contaminated raw material. The newer cell identity technologies such as STR profiling

can identify a human cell to the individual donor level, and the technologies for identifying animal cells (such as CO1 barcoding) have much greater specificity compared with traditional methods. Strategies for cell line engineering have resulted in cell substrates with much greater productivity, and improvements in cloning technology are making it much easier to ensure monoclonality of the production cell.

References

1 International Conference on Harmonisation (ICH) 1997. Q5D. Derivation and characterisation of cell substrates used for production of biotechnological/biological products.

2 Lai T, Yang Y, Ng SK 2013. Advances in mammalian cell line development technologies for recombinant protein production. *Pharmaceuticals* 6:579–603.

3 Sander JD, Joung JK 2014. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nat Biotechnol* 32(4):347–355.

4 Camire J 2000. Chinese hamster ovary cells for the production of recombinant glycoproteins. *Art Sci* 19:150–151.

5 Durocher Y, Butler M 2009. Expressions systems for therapeutic glycoprotein production. *Curr Opin Biotechnol* 20:700–707.

6 Kim TK, Eberwine JH 2010. Mammalian cell transfection: the present and the future. *Anal Bioanal Chem* 397:3173–3178.

7 BioRad Laboratories. Transfection methods overview. www.bio-rad.com/transfection (accessed April 7, 2017).

8 Lee C, Ly C, Sauerwald T, Kelly T, Moore G 2006. High-throughput screening of cell lines expressing monoclonal antibodies. *BioProcess Int* 4:32–35.

9 BioRad Bulletin 10-0503 Rev A 2010. Post-transfection analysis of cells. http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_5969.pdf (accessed April 25, 2017).

10 Yang G, Withers SG 2009. Ultrahigh-throughput FACS-based screening for directed enzyme evolution. *Chembiochem* 10:2704–2715.

11 Black CB, Duensing TD, Trinkle LS, Dunlay RT 2011. Cell-based screening using high-throughput flow cytometry. *Assay Drug Dev Technol* 9:13–20.

12 Dharshanan S, Chong H, Hung CS, Zamrod Z, Kamal N 2011. Rapid automated selection of mammalian cell line secreting high level of humanized monoclonal antibody using Clone Pix FL system and the correlation between exterior median intensity and antibody productivity. *Electron J Biotechnol* 14. 10.2225/vol14-issue2-fulltext-7.

13 Nims RW, Sykes G, Cottrill K, Ikonomi P, Elmore E 2010. Short tandem repeat profiling: part of an overall strategy for reducing the frequency of cell misidentification. *In Vitro Cell Dev Biol Anim* 46:811–819.

14 Capes-Davis A, Theodosopoulos G, Atkin I., Drexler HG, Kohara A, MacLeod RAF, Masters JR, Nakamura Y, Reid YA, Reddel RR, Freshney RI 2010. Check your cultures! A list of cross-contaminated or misidentified cell lines. *Int J Cancer* 127:1–8.

15 Rabenau H, Ohlinger V, Anderson J, Selb B, Cinatl J, Wolf W, Frost J, Mellor P, Doerr HW 1993. Contamination of genetically engineered CHO-cells by epizootic haemorrhagic disease virus (EHDV). *Biologicals* 21:207–214.

16 Burstyn DG 1996. Contamination of genetically engineered Chinese hamster ovary cells. *Dev Biol Stand* 88:199–203.

17 Garnick RL 1996. Experience with viral contamination in cell culture. *Dev Biol Stand* 88:49–56.

18 Oehmig A, Büttner M, Weiland F, Werz W, Bergemann K, Pfaff E 2003. Identification of a calicivirus isolate of unknown origin. *J Gen Virol* 84:2837–2845.

19 Nims R 2011. Adventitious viral contamination of biopharmaceuticals: who is at risk? *BioProcess J* 10:4–10.

20 Moody M, Alves W, Varghese J, Khan F 2011. Mouse minute virus (MMV) contamination—a case study: detection, root cause determination, and corrective actions. *PDA J Pharm Sci Technol* 65:580–588.

21 Potts B 2011. Adventitious agent control and regulations of raw materials used in biopharmaceutical manufacturing. *Am Pharm Rev* 14:76–79.

22 Skrine J 2011. A biotech production facility contamination case study—mouse minute virus. *PDA J Pharm Sci Technol* 65:599–611.

23 Qiu Y, Jones N, Busch M, Pan P, Keegan J, Zhou W, Plavsic M, Hayes M, McPherson JM, Edmunds T, Zhang K, Mattaliano RJ 2013. Identification and quantitation of vesivirus 2117 particles in bioreactor fluids from infected Chinese hamster ovary cell cultures. *Biotechnol Bioeng* 110:1342–1353.

24 Shepherd AJ, Wilson NJ, Smith KT 2003. Characterisation of endogenous retrovirus in rodent cell lines used for production of biologicals. *Biologicals* 31:251–260.

25 International Conference on Harmonisation (ICH) 1997. Q5A(R1). Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin.

26 Code of Federal Regulations (CFR) 2012. Requirements for ingredients of animal origin used for production of biologics. 9 CFR 113.53.

27 European Medicines Agency 2012. Note for Guidance on the use of bovine serum in the manufacture of human biological medicinal products. EMA EMA/CHMP/BWP/457920/2012.

28 European Medicines Agency 2014. Guideline on the use of porcine trypsin used in the manufacture of human biological medicinal products. EMA/CHMP/BWP/814397/2011.

29 World Health Organization (WHO) 2010. Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks. Proposed replacement of TRS 878, Annex 1, 2010. WHO Expert Committee on Biological Standardization, Geneva.

30 European Medicines Agency 2008. Guideline on virus safety evaluation of biotechnological investigational medicinal products. EMEA/CHMP/BWP/398498/2005.

31 International Conference on Harmonisation (ICH) 1996. Q5B. Analysis of the expression construct in cell lines used for production of r-DNA derived protein products.

14

Conduct of Risk Assessments

An Integral Part of Compliance with ICH Q5A and ICH Q5D

Raymond W. Nims

RMC Pharmaceutical Solutions, Inc., Longmont, CO, USA

14.1 Introduction

A common theme for the various ICH quality documents reviewed within this book is the assessment and mitigation of patient safety risk associated with the various types of small molecule and biological products. This assessment of risk is either explicitly discussed within the documents or is implicit in the expectations articulated within the guidance. For example, the establishment of product- and process-related impurity specifications for a given biologicals manufacturing process as described in Q6B [1] is based on the assessment of the safety risk associated with these impurities. In the case of process-related impurities such as residual solvents, the risks may take the form of toxicity, mutagenicity, or carcinogenicity. In the case of product-related impurities (e.g., product variants, aggregates, truncated forms, etc.), the risks may include immunogenicity, toxicity, and other adverse effects. Compliance with the Q6B guidance requires the conduct of various risk assessment analyses that take into account the amounts of the impurities generated, the known or suspected toxicities of the impurities, and the abilities of the manufacturing processes to clear the impurities. This topic is discussed in detail in Chapters 17 and 18 and will not be elaborated further here.

Within the overall process of risk management as defined in ICH Q9 (Quality Risk Management) [2], a key aspect of any risk assessment is an assessment of the likely severity of a given risk event. The Q5A(R1) [3] and Q5D [4] guidance documents are intended to take the form of safety risk/benefit analyses and mitigation of safety risks associated with potential endogenous and adventitious agent contaminants in manufacturing processes.

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In the case of Q5A(R1), risk assessment is discussed in several sections of the document. For instance, section III. C. refers to a risk/benefit analysis for determining acceptability of production cells: "The acceptability of cell lines containing viruses other than endogenous retroviruses will be considered on an individual basis by the regulatory authorities, by taking into account a risk/benefit analysis based on the benefit of the product and its intended clinical use, the nature of the contaminating viruses, their potential for infecting humans or causing disease in humans, the purification process for the product (e.g., viral clearance evaluation data), and the extent of the virus tests conducted on the purified bulk" [3]. This particular risk assessment therefore applies to the relatively unique case of a production cell substrate known to be infected with a virus other than an endogenous retrovirus. Such production cell substrates might be used for subunit vaccine production or to produce gene therapy vectors.

An assessment of the capacity of downstream purification processes for clearing endogenous retroviral particles associated with certain types of production cells (especially human, mouse, and hamster) is another form of risk assessment. This assessment is described in Appendix 5 of Q5A(R1). An example of the process that may be used in conducting this type of risk assessment is described in this chapter.

In Q5D section 2.1.2, the mitigation of the risk of experiencing an adventitious agent contamination during manufacture of a biological is described in the following passage: "Regarding the generation of cell substrates, applicants should provide a thorough discussion of procedures which would provide exposure to infectious agents. Constituents of the culture medium should be described, in particular, information regarding exposure of cells to materials of human or animal origin such as serum, enzymes, hydrolysates, or other living cells. The description should include the source, method of preparation and control, test results, and quality assurance. Relevant literature on these points may be referenced when available. This information will allow a detailed analysis of potential entry routes for adventitious agents from these sources, and will be part of the risk-benefit analysis of the product" [3]. Implementation of the type of risk assessment called for in this section of Q5D may be addressed through two complementary approaches discussed in this chapter: (i) the implementation of a formal and documented animal-derived materials (ADM) assessment program and (ii) the conduct of an overall adventitious agent risk assessment for the manufacturing process.

Taken together, these four types of risk assessment serve different purposes but have in common the goal of providing evidence to the regulatory agencies of thoughtful consideration, by the manufacturer, of various aspects of biologicals production that relate to adventitious agent safety. These types of risk assessment result in written evaluations that can feed directly into the relevant sections of an Investigational New Drug Application (NDA) or Biologics

License Application (BLA) for filing in the United States or a Common Technical Document (CTD) for filing in the United States, the EU, and Japan.

14.2 Conduct of a Risk/Benefit Analysis for the Use of a Cell Substrate Known to Be Infected with a Virus Other Than a Non-endogenous Retrovirus

The cells used to produce biologicals are expected to be free of viruses other than (in the case of rodent cells) endogenous retroviruses. Per Q5A(R1), the "acceptability of cell lines containing viruses other than endogenous retroviruses will be considered on an individual basis by the regulatory authorities, by taking into account a risk/benefit analysis based on the benefit of the product and its intended clinical use, the nature of the contaminating viruses, their potential for infecting humans or causing disease in humans, the purification process for the product (e.g., viral clearance evaluation data), and the extent of the virus tests conducted on the purified bulk" [3].

This particular risk assessment therefore applies to the case of a production cell substrate known to be infected with a virus other than an endogenous retrovirus. Such production cell substrates might be used, for instance, for subunit vaccine production or to produce gene therapy vectors or oncolytic virus preparations. In most cases where the virus will be retained in the final dosage form, such non-endogenous viruses will have been engineered in some manner to attenuate or eliminate their potential infectivity or pathogenicity for human recipients. This might be accomplished through deletion of critical genomic content that is required for infection or pathogenicity or through phenotypic modification such as conferring of temperature sensitivity.

The risk/benefit analysis should contain a discussion of each of the elements specifically called for in the Q5A passage quoted previously. For subunit vaccines and other products that are expected to be virus-free in the final dosage form, there will be increased scrutiny on the validation of the purification processes for the product (e.g., viral clearance validation) and the extent of the virus testing conducted on the purified bulk. For gene therapy vectors or oncolytic virus therapies, where the virus will be expected to be present in the final dosage form, there will be increased scrutiny on the genetic engineering of the viral component of the therapy that has been performed to assure patient safety. In the latter case, the primary arguments may be derived from the construction and characterization of the vector or oncolytic virus itself. An example of a risk assessment for an adeno-associated virus (AAV) vector is available in the literature [5].

Part of the risk analysis, as indicated in the Q5A passage, is the benefit side of the equation (i.e., the benefit of the product and its intended clinical use). The potential for treating debilitating genetic disorders or cancers not readily

treated by existing modalities may provide sufficient benefit to allay the potential risks involved with these types of treatments.

Within the last decade, Vero (*Cercopithecus* monkey) cells used to produce a rotavirus vaccine were discovered to contain infectious porcine circovirus 1 (PCV-1). This followed from the finding of PCV-1 and PCV-2 genomic sequences in the vaccine itself [6]. This finding was subsequently confirmed in press releases from the vaccine manufacturer. The presence of the PCV genomic sequences in the vaccine was attributed to the use of porcine trypsin during the culture of the Vero cell substrate in which the vaccine was manufactured. The trypsin used had been gamma-irradiated to inactivate adventitious viruses prior to use, although gamma irradiation does not appear to be very effective at inactivating this particular small non-enveloped virus [7]. The production cell bank was retrospectively determined to have been infected with PCV-1, and this had gone undetected because assays capable of detecting the virus had not been performed at the time of cell line characterization. During the FDA review of the potential presence of PCV-1 in the rotavirus vaccine, it was determined that there was little evidence to indicate that the virus is infectious or pathogenic for humans. In arriving at this decision, a risk assessment such as that called for in Q5A(R1) in the passage cited previously was prepared [8] by the vaccine manufacturer. This provides a clear case where the risk/benefit assessment concluded that the benefit derived from the therapeutic agent outweighed any specific risk posed by the presence of the adventitious agent.

14.3 Conduct of an Assessment of Viral Clearance Capacity for Endogenous Retrovirus

Certain types of rodent production cells (e.g., mouse, Chinese hamster, and Syrian hamster) are known [9] to harbor within their genomes sequences encoding retroviral particles that may or not be infectious once expressed. It is known that these production cells express such endogenous retroviral particles into the culture medium and therefore into the bulk harvest material used to produce the drug substance. Even in cases such as the Chinese hamster ovary cell where it is known that the expressed retroviral particles are noninfectious [9–12], there is a requirement that the downstream purification process has adequate capacity to clear (remove) such particles. It is required (see Appendix 5 of Q5A(R1)) that the manufacturer perform calculations to determine the theoretical number of such particles that might be present in the volume of bulk drug harvest used to create a final patient dose and to compare this theoretical value with the capacity of the downstream purification processes to clear such particles.

Conduct of this assessment takes the form of the following steps (Figure 14.1): (i) collection of the requisite input information, (ii) performance of the calculation of theoretical maximum viral load (TMVL) in a patient dose, and (iii) addition of a suitable safety factor. The output of the calculation may then be compared with the empirically determined capacity of the downstream processes for clearing retrovirus in order to establish the adequacy of the purification process with respect to retroviral clearance.

As shown in Figure 14.1, there are various inputs that must be obtained prior to completing the calculation. These include the following data, typically obtained from three lots of drug substance manufacture:

a) Mean (or lowest) titer of active (mass/ml in bulk harvest for drug substance)
b) Mean (or highest) viral particle load per ml of the bulk harvest for drug substance determined using transmission electron microscopy
c) Mean (or lowest) overall yield (%) for downstream processing steps

In addition, the risk assessment will require the following information:

d) Mass of active (mg/kg) to be used as a patient dose
e) Information on the overall capacity of the downstream processing steps to clear retrovirus determined through appropriate validation studies performed in duplicate and using a model retrovirus as a challenge virus

Once the aforementioned information has been collected, the calculation of TMVL per patient dose may be performed as follows:

$$\mathrm{TMVL} = \left[\frac{\text{Mass of active in patient dose } (\text{mg/kg}) \times \text{patient weight } (\text{kg})}{\text{Titer of active in bulk harvest } (\text{mg/ml}) \times (\text{downstream yield } (\%)/100\%)} \right] \times \text{Viral particle load } (\text{particles/ml})$$

The patient weight is assumed to be 70 kg for this calculation. The resulting TMVL is in units of particles per dose. This is typically transformed to $\log_{10}$ particles per dose in order to facilitate the next step, which is to add an appropriate safety margin (typically 6 $\log_{10}$).

The value (TMVL (in $\log_{10}$ particles) + 6 $\log_{10}$) may then be compared with the results obtained in duplicate retroviral clearance validation studies. For this comparison, the lowest $\log_{10}$ retroviral clearance value obtained in the two replicate validation studies is typically used. An adequate retroviral clearance capacity in $\log_{10}$ is one that is greater than the TMVL + 6 $\log_{10}$. A summary of this assessment is normally included in CTD Module 3.2.S.2.3 (Control of Materials) and is described in more detail in Module 3.2.S.2.5 (Process Validation and/or Evaluation).

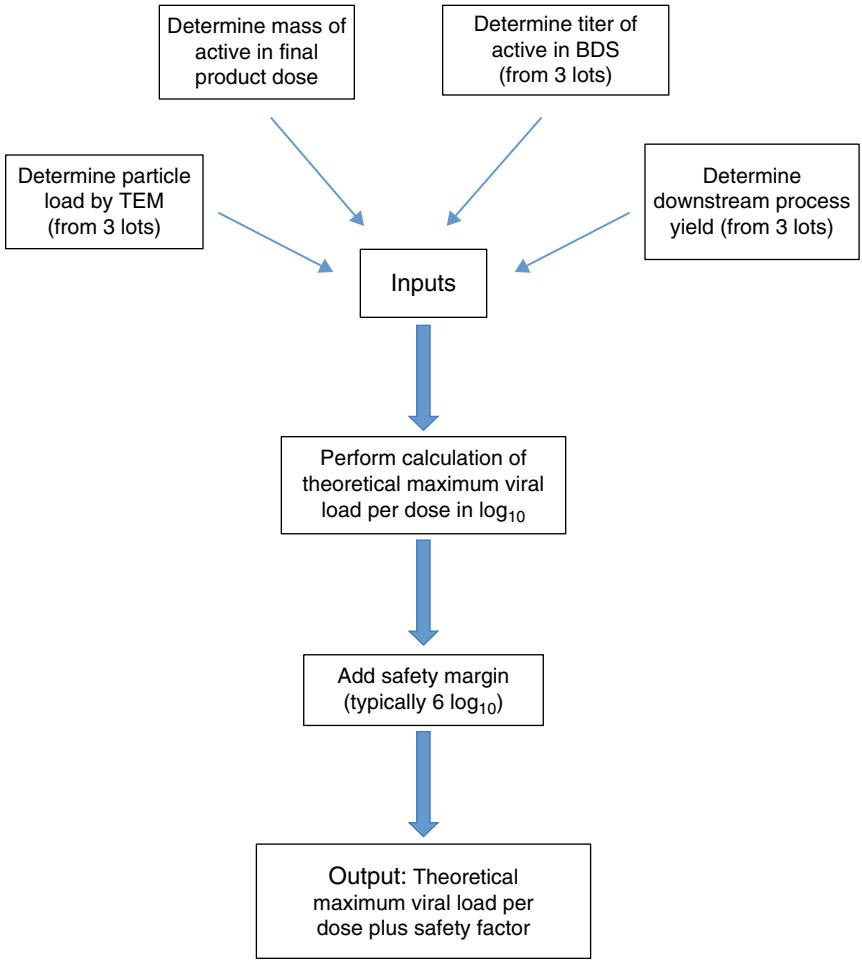

Determine mass of active in final product dose
Determine titer of active in BDS (from 3 lots)
Determine particle load by TEM (from 3 lots)
Determine downstream process yield (from 3 lots)
Inputs
Perform calculation of theoretical maximum viral load per dose in log_{10}
Add safety margin (typically 6 log_{10})
Output: Theoretical maximum viral load per dose plus safety factor

Figure 14.1 Calculation of theoretical maximum viral load per patient dose.

14.4 Implementation of an Animal-Derived Materials Assessment Program

Where it is possible to avoid the use of ADM for biologicals production, it is best to do so. This is because ADM represent potential sources of adventitious agent (e.g., viruses, mollicutes, and transmissible spongiform encephalopathy (TSE, prion) agent) contamination. In regulatory filings it is a requirement that all human-derived and animal-derived materials used during production, or as

excipients in final product formulation, be identified. Such materials are expected to be discussed as part of the overall risk assessment performed for the manufacturing process (see Section 14.5).

If human- or animal-derived materials must be used in a manufacturing process, it is useful to implement an ADM assessment program. Such a program entails the identification of all ADM that are used, the conduct of a risk assessment to determine the relative safety risk posed by such materials, and the documentation of the results of the risk assessments in a form that may directly feed into the relevant sections of regulatory filings (e.g., CTD modules 3.2.A.2 *Adventitious Agents Safety Evaluation*, 3.2.S.2.3 *Control of Materials*, 3.2.P.4.4 *Justification of Specifications*, etc.). These risk assessments may also be used in the performance of the overall adventitious agents risk assessment described in Section 14.5.

Ordinarily, ADM risk assessments are conducted by a team comprising subject matter experts from operations (manufacturing), quality control, and technical services (e.g., virology support). For smaller or virtual companies, this type of support may also be obtained externally through contract service providers. The basic steps involved include:

1) Manufacturing group compiles a list of ADM that are used in manufacture of the product from the bill of materials.
2) Manufacturing group or purchasing group obtains the following information regarding each ADM from the supplier (see also European Pharmacopoeia chapter 5.2.8) [13]:

    a) Animal species of origin
    b) Animal age and tissue of origin (especially important for TSE risk)
    c) Sourcing information relevant to viral/TSE risk (e.g., country of origin)
    d) Traceability of the raw material custody
    e) Collection process (e.g., donor, slaughterhouse)
    f) Conditions and approval of animal slaughter (especially important for TSE risk)
    g) Certificate of suitability (applicable to TSE risk)
    h) Quality control testing, including adventitious agent testing, performed by supplier and described in the certificate of analysis provided for the material
    i) List of viruses, including new and emerging agents, relevant for the given species of origin for the material
    j) Manufacturing process employed in ADM manufacture
    k) Viral/TSE inactivation and/or removal during ADM processing by the supplier or biologicals manufacturer (including treatment approaches such as heat, ultraviolet irradiation, electron beam irradiation, and gamma irradiation)
    l) Risk assessment performed by the ADM supplier/manufacturer

m) Quality audit reports pertaining to the supplier/manufacturer of the ADM
n) Control of supply chain, ADM transportation, and storage conditions

Once the aforementioned information has been collected, the assessment team can meet and perform the formal risk assessment. As shown in Figure 14.2, the risk assessment takes into account the types of adventitious contaminants that might be present in the material given its species/tissue/geographical source of origin, as well as information from the supplier on the processes used to prepare the material. The latter may, depending on the stringency, lessen the risk associated with the use of the material. Adventitious agent testing performed by the ADM supplier, as well as by the biologicals

Raw Material Viral/TSE Risk Assessment

Raw Material:
Supplier:
Supplier Location:
Specification #:
Animal Species of Origin: Animal Tissue of Origin:
Geographical Source of Origin: Certificate of Suitability:
Information Provided by the Supplier:

[Summarise the information provided by the supplier that is relevant to the viral/TSE risk assessment. This may include information regarding the manufacturing process, Quality Control testing information, etc. The information summarised should be substantiated by documentation provided by the supplier and consolidated into a non-revisable controlled document with location cited below]

Viral/TSE Risk Assessment:

[Enter the consensus opinion of the risk assessment team regarding the viral/TSE risk associated with the raw material. Substantiate with literature citations if required]

Executive Summary:

On the basis of the sourcing, processing, and/or testing information provided by the raw material supplier (if applicable, and information regarding the processes used by *[enter your company name]* to manufacture *[enter product name]*), *[enter your company name]* risk assessment team concludes that there is no viral risk associated with *[enter raw material name]*. *[Reiterate the reasons for arriving at this conclusion by pasting text from the above section]*

Literature Cited: *(if applicable, otherwise, remove)*

[Enter literature citation, include a controlled document number for a non-revisable record (pdf) of the article, book chapter, etc.]

Location (electronic) of the information used to perform this assessment:
Assessment performed by risk assessment team on: *[enter date of assessment meeting]*

Figure 14.2 Example of a raw materials assessment tool that may be used as part of an ADM assessment program.

manufacturer upon receipt of the ADM, can also be considered in the assessment of the risk associated with the material. Note that there are specific testing requirements for ADM such as bovine sera [14–17] and porcine trypsin [14, 15, 18]. The manufacturer of the biological may also factor in the stage of manufacture in which the ADM is to be used (e.g., upstream vs. early or late downstream) as well as any processing steps following the use of the ADM that might contribute to the reduction of risk associated with potential contaminants.

If manufacturing or purification processes for the biological itself must be considered in order to justify the acceptability of the ADM risk, then the resulting risk assessment for the ADM must be considered "product specific" and must be documented as such. If the same ADM is subsequently used in the manufacture of a different product, a separate risk assessment will have to be performed taking into account the specifics of that process.

The results of the risk assessment of the ADM should be documented in a risk assessment report that might take the form of the tool shown in Figure 14.2. This should be a version-controlled document signed by the team members conducting the review. Such an assessment is specific to the information used during the assessment, so the report and supporting information must be archived in such a manner that they can be linked. If any of the information used to conduct the risk assessment for an ADM changes materially, the risk assessment will need to be redone.

14.5 Conduct of an Overall Adventitious Agent Safety Assessment for the Manufacturing Process

It is expected that an adventitious agent safety assessment be performed for each biological product manufacturing process. This should take into account the various sources of possible viral, mollicute, or TSE introduction and the controls in place (e.g., sourcing, testing, clearance, manufacturing process design, facility design, gowning, aseptic technique, training) to safeguard the final product. While this is implicit in the language of Q5D section 2.1.2, this type of analysis is also called for in European Pharmacopoeia Chapter 5.1.7 (*Viral Safety*) [19]. The latter guidance provides a greater level of detail on the kinds of factors that should be considered in this type of risk assessment than the ICH document, but it is specific for viruses. The European Pharmacopoeia chapter does, however, represent a good starting place for conducting this overall safety assessment. As with the other types of risk assessment described in this chapter, the overall adventitious agent safety assessment, once completed, can feed directly into the pertinent modules of regulatory filings (e.g., CTD module 3.2.A.2 *Adventitious Agents Safety Evaluation*).

The process for conducting the overall adventitious agent safety assessment for a biological is described in the succeeding text and considers a variety of factors. On the risk side, factors include the use of ADM, the type and history of the production cell, certain aspects of the manufacturing process (e.g., open operations, process interventions, duration of culture, type of cell substrate, etc.), and the manufacturing facility setup. Some of these risks and associated concerns are displayed in Figure 14.3. These risks are mitigated through the ADM risk assessments described in Section 14.4, viral safety testing performed for the ADM by the supplier or the biologicals manufacturer, use of GMP cell banking approaches discussed in Q5D, and viral safety testing of the production cell banks (master, working, and end-of-production cell bank, as described in Q5D). Further useful guidance relating to facilities can be found within various International Organization for Standardization (e.g., standard 14644) [20, 21] and cGMP (e.g., EudraLex, volume 4 part 1 [22] and Code of Federal Regulations [23]) facility requirements. In cases where the nature of the product allows for downstream purification, the validation of the viral clearance capacity of certain purification steps provides additional evidence of risk mitigation. The patient profile and dosing regimen may also be considered during the safety assessment.

The format that the safety assessment takes is not as important as its contents. It should be noted that risk assessments can be executed separately for the various classes of adventitious agents (i.e., mollicute, TSE, virus, etc.), given some of the specifics associated with each of these agents, as opposed to bundling each of these agents into a single risk assessment. The end result might still be one report with individual subsections, with final concluding remarks linking the individual risk assessments together.

The safety assessment report should address the various topics of concern and should be approved and archived as a supporting document, portions of which may feed directly into regulatory filings as discussed previously.

The safety assessment should acknowledge the overriding philosophy of the ICH Q5A/Q5D guidance documents, the essence of which is that the safety of biologicals is based on the following elements:

- Selection and release of raw materials based on prior material and supplier quality approval and qualification testing for adventitious contaminants
- Testing for various adventitious contaminants at appropriate stages of the manufacturing process, including raw materials, starting materials (banks, seeds), and manufacturing intermediates
- Evaluation of viral clearance steps employed in raw material control and testing of the capacity and capability of downstream purification process to clear (remove or inactivate) potential adventitious contaminants in process streams

The risks associated with TSE agents and mollicutes are primarily addressed through sourcing strategies and barrier treatments applied to the ADM that might be used during manufacture of the ADM (described in Section 14.4).

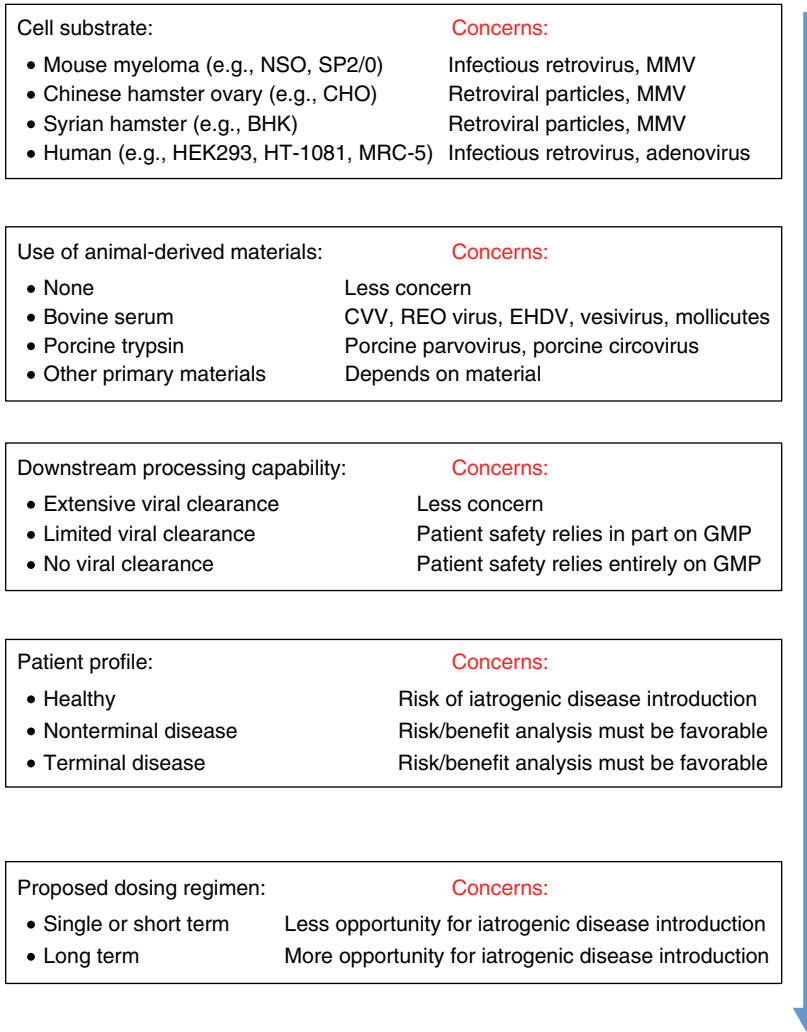


Figure 14.3 Some of the elements of an overall adventitious agent safety assessment for a manufacturing process and associated concerns. The arrow indicates the temporal relationship between the various factors (i.e., upstream manufacturing vs. downstream processing and finally patient profile and dosing).

A risk of viral contamination is inherent in the use of mammalian production cells for biologicals manufacturing and is attributed not only to raw materials that might be used during cell culture but also to the production cells themselves as well as the manufacturing environment. The conduct of the viral safety risk assessment is driven by Q5A(R1) and European Pharmacopoeia <5.1.7> [19].

Per ICH Q5A(R1), "The type and extent of viral tests and viral clearance studies required at different steps of production will depend on various factors and should be considered on a case-by-case and step-by-step basis. The factors that should be taken into account include the extent of cell bank characterisation and qualification, the nature of any viruses detected, culture medium constituents, culture methods, facility and equipment design, the results of viral tests after cell culture, the ability of the process to clear viruses, and the type of product and its intended clinical use" [3].

Also per Q5A (R1), "The approach used by manufacturers in their overall strategy for ensuring viral safety should be explained and justified. In addition to the detailed data which is provided, an overall summary of the viral safety assessment would be useful in facilitating the review by regulatory authorities. This summary should contain a brief description of all aspects of the viral safety studies and strategies used to prevent virus contamination as they pertain to this document" [3].

A framework for the content of such a viral safety risk assessment is provided in European Pharmacopoeia <5.1.7> *Viral Safety* [19]. The factors to be considered include the species and tissue of origin of the production cells and the potential viral contaminants in view of the origin of the production cells, the potential viral contaminants from the manufacturing process (e.g., ADM used and manufacturing process and facility design characteristics), the infectivity and pathogenicity for humans of the potential viral contaminants identified, the amount of bulk harvest used to prepare a human dose, the controls (testing) applied to raw materials and final product, and the capacity and capability of the purification processes to remove or inactivate the potential viral contaminants (see Section 14.3 for the process for evaluating retroviral clearance capability). Some of these factors and associated risks are displayed in Figure 14.3.

An assessment that addresses each of these concerns and also discusses the manufacturing and testing track record for the product (i.e., safety testing results for as many GMP lots of material as have been created and tested) should fulfill the expectations of the regulatory reviewers. Many of these same topics will be addressed in CTD Module 3.2.A.2, so having done this evaluation in advance will better prepare the manufacturer for the preparation of regulatory filings.

14.6 Conclusions

In this chapter, the author has provided examples of the types of risk assessments that may be conducted to satisfy expectations articulated within ICH Q5D and Q5A(R1). These types of risk assessments are different than the tools normally used in risk analysis and management (e.g., failure modes and effects analysis (FMEA), risk matrix analysis, preliminary hazard analysis, fault tree analysis, etc.) insofar as they are focused specifically on the safety risk.

The need for the conduct of the types of risk assessment described here is mentioned within the guidance documents themselves (e.g., Q5A(R1) and Q5D), but the guidance documents provide little detail on the actual implementation of such risk mitigation and characterization activities. The information conveyed in this chapter will, hopefully, clarify the processes that *might be followed* in attempting to meet these regulatory expectations. The completed assessments will facilitate the preparation of regulatory submissions and also will be helpful in satisfying regulatory questions that may come up during facility inspections.

References

1 International Conference on Harmonisation (ICH) 1999. Q6B. Specifications: test procedures and acceptance criteria for biotechnological/biological products.

2 International Conference on Harmonisation (ICH) 2005. Q9. Quality risk management.

3 International Conference on Harmonisation (ICH) 1997. Q5A(R1). Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin.

4 International Conference on Harmonisation (ICH) 1998. Q5D. Derivation and characterisation of cell substrates used for production of biotechnological/biological products.

5 Tenenbaum L, Lehtonen E, Monahan PE 2003. Evaluation of risks related to the use of adeno-associated virus-based vectors. *Curr Gene Ther* 3:545–565.

6 Victoria JG, Wang C, Jones MS, Jaing C, McLoughlin K, Gardner S, Delwart EL 2010. Viral nucleic acids in live-attenuated vaccines: detection of minority variants and an adventitious virus. *J Virol* 84:6033–6040.

7 Plavsic ZM, Bolin S 2001. Resistance of porcine circovirus to gamma irradiation. *BioPharm* 14(4):32–36.

8 Dubin G, Toussaint J-F, Cassart J-P, Howe B, Boyce D, Friedland L, Abu-Elyazeed R, Poncelet S, Han HH, Debrus S 2013. Investigation of a regulatory agency enquiry into potential porcine circovirus type 1 contamination of the human rotavirus vaccine, Rotarix™ Approach and outcome. *Hum Vacc Immunother* 9:2398–2408.

9 Adamson SR 1998. Experiences of virus, retrovirus and retrovirus-like particles in Chinese hamster ovary (CHO) and hybridoma cells used for production of protein therapeutics. *Dev Biol Stand* 93:89–96.

10 Hojman F, Emanoil-Ravier R, Lesser J, Périès J 1989. Biological and molecular characterisation of an endogenous retrovirus present in CHO/HBs—A Chinese hamster cell line. *Dev Biol Stand* 70:195–202.

11 Dinowitz M, Lie YS, Low ML, Lazar R, Fautz C, Potts B, Sernatinger J, Anderson K 1992. Recent studies on retrovirus-like particles in Chinese hamster ovary cells. *Dev Biol Stand* 76:201–207.
12 Shepherd AJ, Wilson NJ, Smith KT 2003. Characterisation of endogenous retrovirus in rodent cell lines used for production of biologicals. *Biologicals* 31:251–260.
13 European Pharmacopoeia Chapter 5.2.8 Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products.
14 Code of Federal Regulations (CFR) 1995. Detection of extraneous viruses by the fluorescent antibody techniques. 9 CFR 113.47
15 Code of Federal Regulations (CFR) 2012. Requirements for ingredients of animal origin used for production of biologics. 9 CFR 113.53.
16 European Medicines Agency 2013. Guideline on the use of bovine serum in the manufacture of human biological medicinal products. EMA/CHMP/BWP/457920/2012 rev. 1.
17 United States Pharmacopeia <1024> Bovine serum.
18 European Medicines Agency 2013. Guideline on the use of porcine trypsin used in the manufacture of human biological medicinal products. EMA/CHMP/BWP/814397/2011.
19 European Pharmacopoeia Chapter 5.1.7. Viral safety.
20 International Organization for Standardization (ISO) Standard 14644: Cleanrooms and Associated Controlled Environments—Part 1: Classification of Air Cleanliness.
21 International Organization for Standardization (ISO) Standard 14644: Cleanrooms and Associated Controlled Environments—Part 4: Design, Construction and Start-up.
22 EudraLex 2014. Volume 4, part 1. Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use.
23 Code of Federal Regulations (CFR) 2015. Current Good Manufacturing Practices. 21 CFR 211.

15

ICH Q5E Comparability of Biotechnological/ Biological Products Subject to Changes in Their Manufacturing Processes

Summary and Analysis of ICH Q5E Guideline

Ramani R. Raghavan[1] *and Robert McCombie*[2]

[1] *Merck & Co., Inc., Rahway, NJ, USA*
[2] *Genentech Inc., San Francisco, CA, USA*

15.1 Introduction

Historically, the quality of a biological product has been closely associated with its manufacturing process. The manufacturing process for a biological product continues to evolve through the different stages of the product life cycle, starting from early development (to support first-in-human clinical studies) to pivotal clinical studies, commercial launch, and postapproval marketing stages. Manufacturers may choose to change the manufacturing process for a given product for many reasons. They include, *inter alia*, a desire to achieve better yields, better product purity via incorporation of newer technologies, increase in scale, or to comply with changes in regulatory requirements. Changes in production methods for a biological product may necessitate an assessment of comparability to ensure that these have not adversely affected the safety, identity, purity, and/or efficacy of the product.

While analytical methodologies used in characterizing protein therapeutics have evolved considerably in the last decade, the manufacturing process is still an important component in defining the product. A good understanding of the product and its manufacturing process, together with a robust control strategy, forms the backbone of a well-characterized biological product that will have consistent quality, safety, and efficacy attributes for every batch of the product manufactured. A robust comparability assessment of the pre- and post-change products will ensure a good understanding of impact of a manufacturing change on the product's safety and efficacy profiles as they relate to patients.

As analytical methodologies evolved, the ability to assess the impact of a change in the manufacturing process increased to the point where it became

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reasonable to consider the concept of comparability. As a result, several regulatory guidance documents were issued by the authorities in the United States and Europe.

The US Food and Drug Administration (FDA) was among the first to provide regulatory guidance to the industry on assessing comparability of biotechnological products. In 1996 and 1997, the FDA published separate guidance documents [1, 2] on assessing comparability of biotechnological products and also created a unique regulatory pathway to submit proposed changes to a biological product via the use of comparability protocols. Submitting a comparability protocol in an application allowed manufacturers not only to gain agreement on what would be necessary to validate a manufacturing change but also to potentially reduce the reporting category for a specific change.

The European Medicines Agency (EMA) published two separate guidelines covering comparability studies needed for manufacturing changes introduced by a manufacturer for a product either before or after the granting of a marketing authorization and for a product claimed to be similar with another one already authorized (biosimilar) [3, 4].

The ICH Q5E guideline [5] concerning the comparability assessment of biotechnological/biological products subject to changes in their manufacturing process came into effect in June 2005. The guideline provides a harmonized approach to performing comparability assessments of biotechnology products after manufacturing process changes are made and assessing the impact of any observed differences in the quality attributes for a product as it relates to the safety and/or efficacy of the product.

15.2 General Principles and Scope of the ICH Q5E Guideline

The guideline provides a uniform set of internationally accepted principles for assessing comparability of biological and biotechnological products before and after changes are made to the manufacturing process for the drug substance or drug product. The term "manufacturing process change" is used within the guideline to delineate changes in the method of manufacture including, but not limited to, changes in starting materials/raw materials, cell culture and purification techniques, and facility and equipment changes that may have an impact on the potential "critical quality attributes" of the product. A critical quality attribute might be defined as a physical, chemical, biological, or microbiological attribute that must fall within a defined range of values to assure that the product will have the desired characteristics of safety, identity, purity, and/or efficacy when administered to the patient. The manufacturer of the product has the ultimate responsibility to demonstrate adequate and appropriate comparability between pre- and post-change products. The principles as put forth in the guideline apply to both marketed products and those that are in development.

Considerations on comparability assessment for products in development are explained further in Section 15.4.9. Included in the scope of the guideline are proteins, polypeptides, and their derivatives produced from recombinant or nonrecombinant cell culture expression systems, and products of which they are components (e.g., protein–drug conjugates). This guideline may also apply to other product types such as proteins and polypeptides isolated from tissues and body fluids; however, it would be prudent to consult with relevant regional regulatory authorities to determine applicability in such cases.

The objective of a comparability exercise is not to prove identity but rather to demonstrate a high degree of similarity between the pre- and post-change products and that the existing knowledge is sufficiently predictive to ensure that any observed differences in the quality attributes have no adverse impact on the safety and efficacy of the drug product. In most cases, existing product and process knowledge assumes critical importance in providing a robust data package to support the comparability assessment of the pre- and post-change products.

Determination of comparability can be viewed as a multi-tiered approach based on a combination of process and product risk assessment, analytical testing, biological assays, and, in some cases, nonclinical and clinical data. An underlying principle of comparability is that under certain conditions, protein products may be considered comparable on the basis of analytical testing results alone. However, the ability to compare biological materials is solely dependent on the tests used, since no single analytical method is able to compare every aspect of protein structure or function. The advantages and disadvantages of any given method depend on the protein property being characterized. Comparison of quality data generated on the pre- and post-change products may include data from routine batch analyses, in-process controls, process validation/evaluation data, product characterization, and stability testing. Where there is adequate assurance of comparability based on analytical studies, further testing of the product through nonclinical and clinical studies may not be warranted. The degree of assurance that product comparability has been established, or whether any observed analytical differences are acceptable, depends on an understanding of the link between physicochemical and biological quality attributes and safety and efficacy, including pharmacokinetics (PK) and mechanism of action. Understanding of these linkages increases as the product progresses through the product development life cycle. However, if the relationship between a particular quality attribute and safety and efficacy of the product has not been clearly established and differences between quality attributes of the pre- and post-change products are seen, it may be appropriate to also include nonclinical and/or clinical studies in the comparability assessment.

Robust risk assessments must be performed that will allow identification of the likely impact of a given manufacturing process change on the product. In addition, predefining appropriate criteria to demonstrate a highly similar post-change product will lead to more objective assessments of comparability

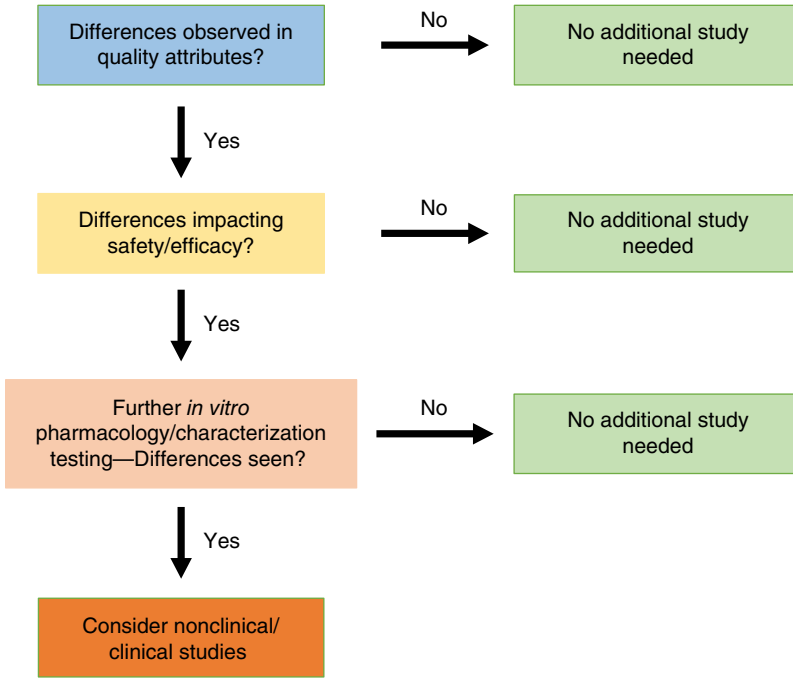


Figure 15.1 Comparability assessment; possible outcomes.

when comparing quality data generated for pre- and post-change products. Comparability assessments performed in this manner generally lead to one of the following outcomes (Figure 15.1):

- The pre- and post-change products are highly similar and are therefore considered to be comparable. No adverse impact on safety or efficacy profiles is foreseen.
- Pre- and post-change products appear highly similar; however analytical procedures used in the assessment did not adequately discern relevant differences that may impact the safety and efficacy of the product. Therefore, further characterization testing or nonclinical and clinical testing to ascertain comparability is warranted.
- Products appear highly similar but some differences in the quality attributes have been observed. However, based on prior knowledge, manufacturing experience, relevant risk assessments, and literature data, no adverse impact on the safety and efficacy profiles is expected. Therefore, the pre- and post-change products can be considered comparable.
- Products appear highly similar; however, some differences in quality attributes have been observed, and a potential adverse impact to safety and efficacy

profiles cannot be ruled out. In this case, nonclinical and clinical evaluation may be warranted.

- Differences in quality attributes observed between pre- and post-change products are so significant that the products cannot be considered highly similar and therefore are not comparable. This scenario is outside the scope of the ICH Q5E comparability guideline and will not be discussed in this chapter.

15.3 Manufacturing Process

When a manufacturing process change is made to a product, the impact of that change on the product depends on several factors, including the type and complexity of the change and product characteristics. A comparability testing plan for a pre- and post-change product and the approach for determining impact rely heavily on the manufacturer's knowledge and experience with the product and its manufacturing process. A well-defined manufacturing process and associated process controls will ensure that every batch of the product produced is of high quality and consistently meets a predefined set of acceptance criteria for that product. Process controls in the post-change process should provide similar or greater assurance of product quality when compared with the original process.

The potential effects of the planned change on downstream steps need to be carefully considered, including possible effects on corresponding quality parameters (e.g., in-process controls/specification, hold times, operating limits, and validation/evaluation). This will help in the determination of appropriate tests and corresponding acceptance criteria for the comparability exercise. To support the process changes and corresponding comparability assessment of the pre- and post-change products, data from commercial-scale batches is preferred and often required. It is often useful to demonstrate, for example, that specific intermediates are comparable or that the modified process has the capability to provide appropriate levels of removal of process- and product-related impurities (see also Chapters 17 and 18), including any that are newly introduced by the process change.

The process assessment for a specific product and process should consider such factors as the criticality of the process step and proposed change, the location of the change in the process and potential for effects on other process steps, and the type and extent of the change. The body of knowledge that helps in the assessment includes, among others, process platform experience, data from process development studies, experience with earlier process changes specific to that product, and literature data. The characteristics that might be affected often may be predicted based on the body of knowledge that exists and a robust understanding of the process change with respect to that product. If changes in product characteristics are anticipated, methods to monitor those

attributes must be selected and the appropriate levels of validation and precision should be assured. At times, no characteristic changes are anticipated, but testing should not be overlooked; a demonstration that critical attributes have been preserved is also important.

The modified process steps should be reevaluated and/or validated as appropriate. The in-process controls, including critical control points and in-process testing, should ensure that the post-change process is well controlled and maintains the quality of the product. For a simple process change, reevaluation or validation could be limited to the affected process step, provided there is no evidence to indicate that there is impact on the performance of downstream (i.e., purification) process steps or on the quality of the intermediates resulting from subsequent steps. However, for complex process changes that affect more than a single step, more extensive analysis of the change and resultant validation may be appropriate. An appropriate number of post-change batches should be analyzed to demonstrate consistent process performance. The number of batches required to establish comparability should be justified and may be based on a number of factors, such as process capability, process and product knowledge, platform process knowledge, and manufacturing facility history. The regulatory submission describing the process change should include a side-by-side description that summarizes the pre- and the post-change manufacturing processes and clearly delineates the process modifications and corresponding controls.

Demonstration of the state of control for the changed manufacturing process may include, but is not limited to, the following:

- Establishment of appropriate specifications for raw materials, starting materials, and reagents
- Appropriate bioburden and/or viral safety testing of the post-change cell banks and cells at the limit of *in vitro* cell age for production
- Adventitious agent clearance
- Removal of product- and process-related impurities, such as residual host cell DNA and proteins
- Similarity or justified improvement of purity levels (e.g., reduction in amount of host cell protein).

15.4 Determination of Comparability: Methods and Considerations

15.4.1 Development of the Comparability Program

The goal of the comparability exercise is to ascertain that the pre- and post-change drug substances and/or drug products are comparable in terms of quality, safety, and efficacy. This goal necessitates that the product be carefully

Table 15.1 Hierarchy of comparability testing.

Category	Testing (determined on a case-by-case basis)
A – Basic package	1) Release specification testing 2) Extended characterization of variants and impurities 3) Accelerated degradation/stress testing
B – Biological characterization	1) *In vitro* functional bioassays and binding studies 2) Real-time and accelerated stability
C – Animal PK/PD	1) Rodent PK 2) Primate PK/PD
D – Human bioequivalence	Direct comparison of pre- and post-change product
E – Human clinical trials	To confirm safety, efficacy, and/or immunogenicity

evaluated at the process step that is most appropriate to detect a change in the relevant quality attributes and, potentially, at multiple stages during manufacture of the product. Therefore, in some cases it may be appropriate to collect data on both drug substance and drug product to establish comparability even though all process changes may have occurred only during manufacture of the drug substance. A comparability protocol should be developed based on the potential impact of the change on quality, safety, and efficacy of the product, the stage of development, and the extent of product and process knowledge and experience with the product. There is a hierarchy to the aspects included in the comparability assessments, starting with analytical and process comparability, animal PK/pharmacodynamics (PD) and toxicology studies, and, finally, adequately powered human bioequivalence and clinical trials, in that order (Table 15.1) [6].

In all evaluations, analytical comparability data are central and often determine the extent to which additional data need to be developed for demonstration of comparability. Accelerated or stress stability studies provide valuable insight into potential product differences in the degradation pathways of the product and, hence, potential differences in the product-related substances and process-related impurities.

Many manufacturing process changes, although perceived to be minor in nature, could actually have an adverse impact on the quality of a product and therefore its safety and efficacy characteristics. For example, variability in glycosylation patterns has been observed in CHO-derived humanized monoclonal antibodies due to raw material changes or changes in unit operation materials of construction. Such changes may lead to subtle differences in leachable trace metal content, and some trace metals are known to impact posttranslational modification during cell culture [7]. For example, the

available iron and manganese present in cell culture media are known to impact glycosylation [8].

In most cases, pre- and post-change comparabilities can be established based on quality studies alone, but sometimes these may have to be supported by nonclinical or clinical comparability bridging studies. The data package needed to demonstrate comparability will depend on several factors:

- The production step where the change is introduced and the complexity of the change
- The extent of available historical data, including product and process knowledge and manufacturing experience that can be used to evaluate the impact of the change on product quality (including physicochemical and biological properties)
- Availability of suitable analytical techniques that are able to reliably discern any differences in the product that may result from the manufacturing process changes
- The relationship between quality attributes and safety and efficacy, based on overall nonclinical and clinical experience

15.4.2 Establishing Comparability Acceptance Criteria

Where formal comparability assessments are conducted, comparability acceptance criteria must be predefined. The number of criteria may be relatively few during early clinical development and may have broad acceptable ranges, commensurate with the amount of product and process knowledge available on which to base an assessment of what is important to efficacy of the product. As experience with the product and process increases through clinical development, the number of applied tests will generally increase and the associated acceptance criteria will tighten in range. In general, acceptance criteria should be based on existing batch and characterization data and should also take into account process and method variability.

If attributes of the molecule are sufficiently understood with respect to safety and efficacy, establishment of acceptance ranges can reflect this knowledge. For instance, broader acceptance ranges may be more appropriate for a variant known not to affect efficacy than for a variant with either a known link to efficacy or a variant for which the impact of change with respect to efficacy is not known. This knowledge of product variants need not always come from the protein under investigation but may come from other related molecules, for example, within the same therapeutic platform, where justified.

Other important aspects to consider when setting comparability acceptance criteria include the stage of product development, protection of the patient, dosing studies, therapeutic indication, treatment population, and prior clinical exposure to the attribute level.

Acceptance criteria should not be based solely on statistical analysis of historical process batch data without an understanding of the nonclinical or

clinical context of the product or indication, analytical method capability, or process capability.

There may be cases for which minor differences occur between pre- and post-change products although all predefined acceptance criteria are met, and it can be determined there is no negative impact to product quality. In such cases, any claim of comparability must be sufficiently supported with reference to the impacted product attributes and any known links to impact on safety and/or efficacy. This is especially true where differences occur that were not expected or defined upfront in the comparability protocol. At the same time, failing an acceptance criterion or acknowledging that the product will not be analytically comparable does not always imply failure of comparability. If it can be clearly demonstrated that there is no negative impact to safety or efficacy, the outcome of the comparability exercise might still be successful. An important aspect to note is that failure to demonstrate comparability includes cases in which an *unexpected improvement* in product quality results from the change.

Comparability acceptance criteria may be assigned to methods using a broad range of approaches including, but not limited to:

- Setting acceptance criteria based on the product specification
- Using statistical approaches based on historical data (such as tolerance intervals [9] or equivalence approach [10])
- Graphical trending methods based on test method capability
- Any combination of these, as appropriate for the attributes in question

In addition to these techniques, it is common to include direct side-by-side comparison of pre- and post-change batches of product using batch release methods, extended characterization methods, and comparative accelerated or stress stability samples. Chromatograms, electropherograms, or spectral data may also be compared with the reference standard and/or pre-change material for assessment of process and product consistency.

15.4.3 Acceptance Criteria Taken from the Specification

Using only the pre-change product specification as acceptance criteria for assessing comparability may be sufficient for comparability evaluation of process changes occurring during early product development. For late-phase or postapproval changes, the specification may be used as acceptance criteria for quality attributes for which there is a scientifically justified rationale that these are unlikely to be impacted by the change. This might be established, for example, through a formal process risk assessment that concludes that the change is focused in nature and unlikely to impact certain quality attributes or affect steps further downstream. Use of product specifications as comparability acceptance criteria may also be acceptable for "obligatory" quality attributes that are either defined by national pharmacopoeia or specified by authority

guidance (e.g., appearance, color, clarity, identity), are arbitrary or formulation related (pH, osmolality), or are facility- or environment-related and linked to general safety (e.g., endotoxin, bioburden).

15.4.4 Statistical Approaches to Setting Acceptance Criteria

Demonstration of comparability using a tolerance interval (TI) approach requires that each individual post-change result falls within a predefined tolerance interval. For demonstration of process comparability, a 95/99 TI is often used. This describes the range (or interval) within which there is 95% confidence that 99% of the test results will fall. When this approach is used, the acceptance criterion range is typically based on a TI centered about the mean historical value from the pre-change manufacturing process.

A statistical test of equivalence is used to compare the means of the test results of pre- and post-change products. The means are evaluated for a difference that might be considered practically important for any impact to product quality. This approach is especially useful when comparing rates of change for pre- and post-change products placed on side-by-side accelerated or stress stability studies [10].

15.4.5 Case Study 1: Targeted Comparability of Drug Substance Manufacturing Processes Following Minor Process Change

This comparability case study concerns a minor corrective modification to a commercial monoclonal antibody drug substance manufacturing process. This change was required to bring the drug substance back into the historical manufacturing trend following a vendor-initiated raw material change, which resulted in a shift of the drug substance glycosylation pattern. The sponsor evaluated the potential impact of the proposed corrective process modification, which comprised a change to the implicated raw material and additional trace element supplementation of cell culture media. The initial evaluation of the change identified that, in addition to process validation, a comparability protocol was required with the objective of establishing that following the corrective modification, the drug substance would be comparable with the pre-change drug substance. Specifically, the comparability exercise was intended to demonstrate that there was no impact on particular product characteristics or on the product- and process-related impurity profiles. This objective was achieved by executing a targeted analytical product comparability program in which the extended product characterization testing was focused on the attributes that might potentially be impacted by the corrective process modification.

Three post-change drug substance batches were planned to establish comparability with the pre-change drug substance and to enable process verification (commercial-scale process validation batches). To select and justify which tests

to include in the comparability assessment, the sponsor leveraged extensive product-specific and platform process knowledge to assess the likely impact of the process changes. This product-specific knowledge base included data from previous process site and scale transfers, extensive small-scale process characterization studies, and real-time and accelerated stability studies. Together, these data were input to a process assessment risk assessment that evaluated the potential impact of corrective process modification on the drug substance quality attributes and impurity profile. The assessment concluded that the process change would likely only impact the glycosylation pattern, as intended.

Additionally, the process risk assessment informed and justified which tests to include in the comparability protocol, due to residual risk of impact to product quality. The tests selected included all the drug substance release tests. In addition, extended analytical characterization focused on select product- and process-related impurity attributes, and real-time and accelerated drug substance stability studies were included in the protocol. The comparability protocol acceptance criteria were largely set based on the outcome of the risk assessment. The drug substance release test acceptance criteria were taken from the drug substance release specification, with a few justified exceptions. Based on the risk assessment, several release test results were identified as presenting a higher risk of being out of the historical trend, and therefore the comparability acceptance criteria were in these cases set tighter than the release specification, using a 95/99 TI approach. The data used to establish the 95/99 TI were from batches manufactured prior to the observed glycosylation pattern shift (i.e., prior to the vendor-initiated raw material change). Additional qualitative acceptance criteria were also included, such as a requirement that no new peaks be observed in the process chromatography profiles. The comparability assessment tests and associated acceptance criteria for this case study are described in Table 15.2.

Three corrective modification batches were manufactured to verify process validation and establish comparability according to the protocol. With respect to cell culture and purification performance, impurity clearance, and extended characterization methods previously described, the three batches met all predefined comparability acceptance. Overall, comparability of drug substance manufactured with the corrective process modification to drug substance manufactured prior to the observed glycosylation pattern shift was demonstrated, and the process modification was successfully registered and implemented in commercial production.

15.4.6 Analytical Methodology

Due to the complexity of therapeutic proteins, analysis of primary structure, posttranslational modifications, and other molecular variants may reveal subtle differences between the products manufactured before and after a process

Table 15.2 Case study 1: comparability protocol tests and acceptance criteria.

Parameter/aspect	Product attribute comparability assessment acceptance criteria
Drug substance release tests	The comparability batches must meet the current drug substance release specification
Higher risk drug substance release tests[a]	Potency assay: • Acceptable range set using 95/99 TI HPLC method 1: • Percent area peak A: acceptable range set using 95/99 TI % • Percent area peak B: acceptable range set using 95/99 TI % HPLC method 2: • Percent area peak A: acceptable range set using 95/99 TI %
Targeted extended characterization	HPLC Glycan method: • Comparable elution profiles between post-change and pre-change drug substance • Individual relevant glycan species percent area are within 95/99 TI *In vitro* antibody-dependent cellular cytotoxicity (ADCC) assay: • Acceptable range set using 95/99 TI %
Process-related impurities (HCP, DNA, and protein A ligand)	The comparability batches must meet the following acceptance criteria at the appropriate justified unit operation pool: CHO host cell protein (HCP) (ng/mg): • Acceptable range set using 95/99 TI % CHO host cell DNA (pg/mg): • Acceptable range set using 95/99 TI % Protein A ligand (ng/mg): • Acceptable range set using 95/99 TI %
Real-time stability and accelerated stability studies	A single comparability batch will be placed at the accelerated stability condition for 3 months and at the real-time storage condition for the registered drug substance shelf life. The acceptance criteria for the stability studies were set on the established commercial end-of-shelf-life specification

[a] Identified as higher risk of being impacted by the process modification during the risk assessment.

change that may not be evident solely from lot release assays. Therefore, it is important to consider inclusion of extended biochemical characterization techniques in the evaluation of product comparability in order to evaluate these subtle potential differences. The battery of tests for a comparability study should be selected and optimized to maximize the potential for detecting

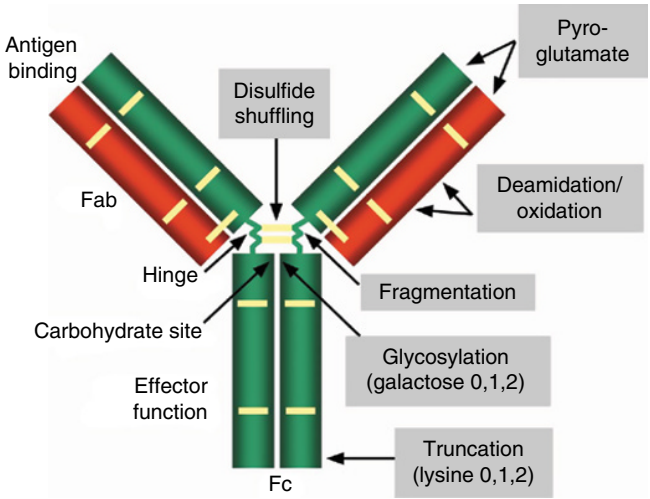


Figure 15.2 Typical forms of protein heterogeneity [11].

relevant differences in the quality attributes of the product resulting from the proposed manufacturing process change. To address the full range of physicochemical and biological properties, it may be appropriate to use orthogonal methodologies to evaluate the same quality attribute (e.g., molecular weight, impurities, secondary/tertiary structures). Selection of analytical methods and acceptance criteria that will be applied to demonstrate comparability can be the most difficult step in a comparability exercise due to limitations of the assays and complexity of some products due to molecular heterogeneity (Figure 15.2) [11, 12].

At the start of a comparability assessment, it is important to determine whether the existing methods remain appropriate for their intended use or if any modifications or new methods are needed. For example, a manufacturing process change could result in a different impurity profile in the host cell proteins. The original analytical method used to quantitate these impurities needs to be confirmed as suitable for its intended purposes as in some cases further modification may be needed. Use of validated methods in characterization studies is not an explicit regulatory requirement per ICH; however, the test methods used must be scientifically sound and provide consistent, reliable results. Validation practices consistent with ICH guidelines (Q2(R1) [13], Q5C [14], Q6B [15]) must be followed for analytical methods used to test and release product (batch release) and to monitor product stability.

The comparability exercise should include a side-by-side analytical profile comparison of the pre- and post-change products that demonstrates consistency with respect to the selected characterization tests and process attributes

(such as cell culture performance, purification process yields, levels of impurities, and other relevant quality criteria).

Analytical methods are often optimized or replaced during product development. It is important to note that where acceptance criteria are based on statistical analysis of historical batch data generated using a previous method (e.g., a TI approach), the comparability protocol should account *a priori* for any data shifts or differences these method changes may introduce.

15.4.7 Case Study 2: Comparability of Drug Substance Following Route of Administration Change

Comparability case study 2 concerns development of a subcutaneous (SC) dosage form of a commercially available intravenously (IV) administered monoclonal antibody. Development of the new dosage form required changes to the product formulation and drug product manufacturing process and also to the drug substance manufacturing process. With the exception of the deliberately changed formulation-related attributes, the SC drug substance and drug product specifications remained largely identical to the IV drug substance and drug product specifications. Due to the new route of administration, strength, and formulation, health authorities required pivotal clinical trials to demonstrate clinical safety and efficacy of the new SC drug product. As a result of these extensive clinical trials, the sponsor determined that a broad comparability assessment was not required between the SC and IV substances and/or products. The SC drug substance used for fill/ finish of the drug product used in the clinical studies was manufactured at a dedicated clinical material manufacturing site. Following successful clinical studies, the drug substance manufacturing process was transferred to the intended commercial manufacturing site, which already manufactured the registered IV drug substance. The manufacturing process site transfer involved minimal changes to the manufacturing process other than for minor facility fit and to the scale of manufacture.

To enable successful registration of the SC dosage form, the sponsor determined that is was necessary to demonstrate that SC drug substance manufactured at the commercial site was comparable to SC drug substance manufactured at the clinical site. Therefore, three SC drug substance batches produced at the commercial site were compared with three SC drug substance batches produced at the clinical site along with comparison to the reference standard, which was produced from drug substance manufactured via the IV drug substance manufacturing process.

Following a risk assessment of the potential effects of the site transfer, acknowledging the minor nature of the changes to the drug substance manufacturing process operations, the tests to establish comparability were chosen based upon their ability to confirm the antibody's primary structure, N-linked glycosylation pattern, product-related variants, biological activity, and

higher-order structure. To this end, the following tests were used to evaluate these attributes:

- The primary structure of the drug substance was assessed by electrospray mass spectroscopic (ES-MS) analysis of reduced drug substance and liquid chromatography–mass spectroscopic (LC–MS) analysis of the antibody tryptic maps. Batch-to-batch consistency was assessed using LC–MS analysis, through a comparison of the chromatographic profiles and a comparison of the levels of oxidation and deamidation in the drug substance batches.
- The distribution of the drug substance size variants was assessed using size-exclusion chromatography and capillary electrophoresis. Batch-to-batch consistency was assessed through a comparison of the electrophoretic and chromatographic profiles and a comparison of the distribution of size variants obtained by integration of the electropherograms.
- The distribution of the drug substance charge variants was assessed using ion-exchange chromatography and isoelectric focusing. Batch-to-batch consistency was visually assessed through a comparison of the chromatographic and electrophoretic profiles and a comparison of the distribution of charge variants obtained by integration of the chromatograms and electropherograms.
- The distribution of the antibody *N*-linked oligosaccharides was assessed through chromatographic analysis of labeled glycans. Batch-to-batch consistency was assessed through a comparison of the chromatographic profiles and a comparison of the distribution of oligosaccharides obtained by integration of the chromatograms.
- The biological activity and binding of the antibody was assessed using the potency bioassay, and surface plasmon resonance analysis was used to assess the binding of the drug substance to the target receptor. Batch-to-batch consistency was assessed by comparing the drug substance activity in both assays.
- The higher-order structure of the antibody was investigated using circular dichroism spectroscopy. Spectral profiles were used to assess batch-to-batch consistency.

In addition to establishing analytical comparability of product-related species and impurities, an assessment was made to establish comparability of the manufacturing process in terms of process-related impurities and clearance of raw materials. Commercial SC drug substance batches were evaluated with respect to the removal of process-related impurities such as host cell-related protein and DNA- and process-related impurities (e.g., cell-selective pressure reagents, cell culture media components, antifoam, chromatographic ligands, and organic solvents).

Data from these analyses confirmed that binding of the antibody to the target receptor occurs in a dose-dependent manner. It was determined from these

analyses that commercial drug substance batches, clinical drug substance batches, and reference standard were comparable.

15.4.8 Characterization of the Product

A pre- and post-change comparability assessment of a manufacturing process change that has a potential to impact quality attributes must include product characterization testing. The extent of characterization testing depends on many factors including the product complexity, development phase, and historical data reflecting a good understanding of the product and process. Manufacturing process changes (e.g., in cell culture and purification processes) can result in significant differences in the physicochemical properties of the protein such as amino acid sequence or posttranslational modifications. These changes can alter the purity and impurities in a recombinant protein therapeutic product and thereby may impact the product's clinical efficacy and safety including causing the production of undesirable side effects and immunogenicity. When a manufacturing process change has been made, characterization testing of the post-change product is warranted (all or a subset of tests submitted in the marketing application) to compare any changes in the quality attributes and, thus, on the product's safety and efficacy profiles. For a process change that results in a product with a different characterization profile from the material used in nonclinical and clinical studies or other appropriate representative materials (e.g., reference materials, marketed batches), the significance of these changes should be appropriately evaluated. Characterization data of pivotal clinical trial batches can provide a useful point of reference for subsequent comparability exercises.

15.4.8.1 Physicochemical Properties

A comparability study involving pre- and post-change products should be designed and conducted in consideration of the principles articulated in the ICH Q6B guideline in relation to the concept of the desired product and its variants. A physicochemical characterization program includes, at a minimum, a determination of the composition, physical properties, and primary structure of the desired product. Proteins have an inherent degree of structural heterogeneity due to the biosynthetic processes used by living organisms to produce them. The desired product can therefore be a mixture of anticipated posttranslationally modified forms (e.g., glycoforms). The comparability assessment should consider the complexity of the protein with respect to the degree of molecular heterogeneity. Variability of monoclonal antibodies can occur on the amino acid backbone or to the carbohydrate moiety of glycosylated proteins (Figure 15.2). It may be useful to perform a risk assessment to understand which variants may be impacted by the change and to allow for a focused comparability assessment. Protein heterogeneity can be attributed to disulfide

bond shuffling, deamidation, methionine oxidation, cyclization of N-terminal glutamine residues to pyroglutamate, and loss of C-terminal lysine [11] (Figure 15.2). Changes in the upstream cell culture processes can induce changes in posttranslational modification of therapeutic proteins. These can result in differential addition of glycan structures such as fucose, alternative mannose branching linkages, galactose residues (termed G0, G1, G2 molecular species), and sialylated moieties.

Changes to higher-order structure of protein products (secondary, tertiary, and quaternary structures) can be induced as a result of manufacturing process changes. The comparability exercise following a manufacturing process change should attempt to determine that higher-order structure (secondary, tertiary, and quaternary structure) is maintained in the post-change product.

15.4.8.2 Changes in Biological Activity

Assessment of biological properties is an essential step in establishing a complete characterization profile of a protein therapeutic product. Biological assay results can help confirm the product quality attributes that are useful for characterization and batch analysis and, in some cases, can serve as a link to clinical activity. As we have seen previously, manufacturing process changes can cause changes in the physicochemical and structural attributes of a protein therapeutic product that, in turn, can affect the potency of the product. Such changes can occur directly through altering the biological activity of the protein, or indirectly by altering the PK or bioavailability of the protein, through changes in glycosylation. For example, removal of total sialic acid content of a glycoprotein by about 20% can result in a loss of 50% or more in the amount of circulating protein [16].

Any limitations of the biological assays, such as high variability, that might prevent detection of differences that occur due to the manufacturing process change should be carefully considered as part of the comparability exercise. For some monoclonal antibody products, monitoring carbohydrate heterogeneity throughout product manufacturing and during comparability assessment is critical because differential sialylation, galactosylation, and fucosylation can affect clinical function, including complement-dependent cytotoxicity (CDC), ADCC, PK, and antigen binding. Biological assays often complement physicochemical testing as, for example, surrogate tests for higher-order structure determination. In such cases, the use of a relevant biological assay with appropriate precision and accuracy might provide valuable information regarding the relationship between a change in specific higher-order structure and the manufacturing process change. Where physicochemical or biological assays are inadequate in confirming that the higher-order structure of the pre-change product is maintained, a nonclinical or clinical study may be warranted.

The design of the comparability study should seek to evaluate all relevant functional activities. Thus, for products with multiple biological activities, a

set of relevant functional assays designed to evaluate the range of activities may be appropriate. When one or more of the multiple activities are not sufficiently correlated with clinical safety or efficacy or when the mechanism of action is not understood, evidence should be provided that establishes that nonclinical or clinical activity is not compromised in the post-change product.

15.4.8.3 Immunological Properties

A change in immunogenic potential that may be triggered due to a manufacturing process change has to be critically evaluated. This characterization should include an assessment of molecular variants or aggregates that are known or likely to be a concern, either from direct clinical experience with that molecule or from the literature. These might include aggregated species for antibodies or antibody-based products. Confirmation should be provided that the pre- and post-change products are comparable for these specific attributes of immunologic concern.

15.4.8.4 Purity, Impurities, and Contaminants

Differences in the purity and impurity profiles for the post-change product have to be carefully evaluated for potential impact on safety and efficacy. When the change results in the appearance of new impurities, the new impurities must be identified and appropriately characterized. In some cases, nonclinical and clinical testing may also be warranted, based on the amount and type of impurities, to confirm that there are no adverse impacts on the safety or efficacy of the drug product. In general, purity and/or impurity levels should be maintained between the pre- and post-change products. Where a manufacturing process change was made to specifically improve an impurity profile, the expected differences for the impacted impurity should be clearly identified and justified in the comparability protocol, together with predefined acceptance criteria.

15.4.8.5 Specifications

The ICH Q6B guideline defines a specification as a list of tests, references to analytical procedures, with appropriate acceptance criteria that are numerical limits, ranges, or other criteria for the tests described. Testing against the specification serves to confirm routine quality of the product that has been manufactured per established manufacturing procedures. The development of analytical data as part of the comparability study should include a comparison of release testing results for the pre- and post-change products. While these results do provide a level of understanding regarding product quality, they may not be adequate in assessing the full impact of the manufacturing process change.

The comparability exercise conducted after a manufacturing process change should also assess the adequacy of the specification in ensuring product quality. Addition of a test, or the deletion or modification of an existing test, may be

needed in cases where the previous test is no longer relevant for routine batch analysis of the post-change product. For example, removal of bovine serum from the upstream cell culture process would obviate the need for certain tests (e.g., freedom from bovine viruses) in the specification or a new test that may be required to quantitate new impurities that may be formed following the manufacturing process change. Results that are within the established acceptance criteria but outside historical manufacturing control trends might suggest product differences that warrant additional study or analysis. Generally, widening of acceptance criteria in a release specification is not considered appropriate unless justified.

15.4.8.6 Stability

Changes to a bio-manufacturing process could alter protein structure and purity. These changes should be evaluated for impact on stability [14], since proteins are frequently sensitive to changes such as those made to buffer composition, processing and holding conditions, and use of organic solvents. A side-by-side stability study comparing the pre- and post-change products should be considered as part of the comparability exercise. Stability studies can be complementary to product characterization studies as they can often detect subtle differences that are not readily detectable through characterization studies. For example, the presence of trace amounts of a protease might only be detected by product degradation that occurs over an extended time period. In some cases, divalent ions leached from the container/closure system may impact the stability profile because of the activation of trace proteases not detected in stability studies of the pre-change product [17]. Real-time/real-temperature stability studies offer valuable clues on the impact of a manufacturing change on the stability of the post-change product and should therefore be initiated, as appropriate. Some changes to a drug substance manufacturing process may affect the drug product. In such cases, the stability evaluation should include a separate drug product stability study to determine impact to the drug product.

Accelerated and stress stability studies can be very useful in elucidating differences in the degradation profile of the post-change product and should be included in the comparability assessment. ICH Q5E states that "any change with the potential to alter protein structure or purity and impurity profiles should be evaluated for its impact on stability, since proteins are frequently sensitive to such changes." [5] Generally speaking, forced degradation conditions may not represent accelerated degradation pathways that may be observed under recommended storage conditions but are useful in detecting differences between changed products. Therefore, forced degradation studies should not be used to quantitatively estimate potential differences in shelf life between changed products that might apply to the recommended storage condition.

Stress conditions used should cause measurable degradation in a reasonable time frame to allow evaluation of degradation rates and any degradation products.

15.4.9 Comparability Assessments for Products in Development

As development of the product progresses, multiple changes to the manufacturing process typically occur that could impact drug product quality, safety, and efficacy. Comparability assessments during development stages yield data to demonstrate that the nonclinical and clinical data generated with the pre-change product are applicable to the post-change product in order to support further development and/or the marketing authorization. Comparability studies conducted for products in development are influenced by factors such as the stage of product development, the availability of validated analytical procedures, and the extent of product and process knowledge. In general, the depth and extent of a comparability exercise is commensurate with the stage of clinical development (refer to Figure 15.3).

During the early phases of product development, comparability testing is generally not as extensive as there is a limited amount of available product knowledge. As development progresses, more manufacturing experience and

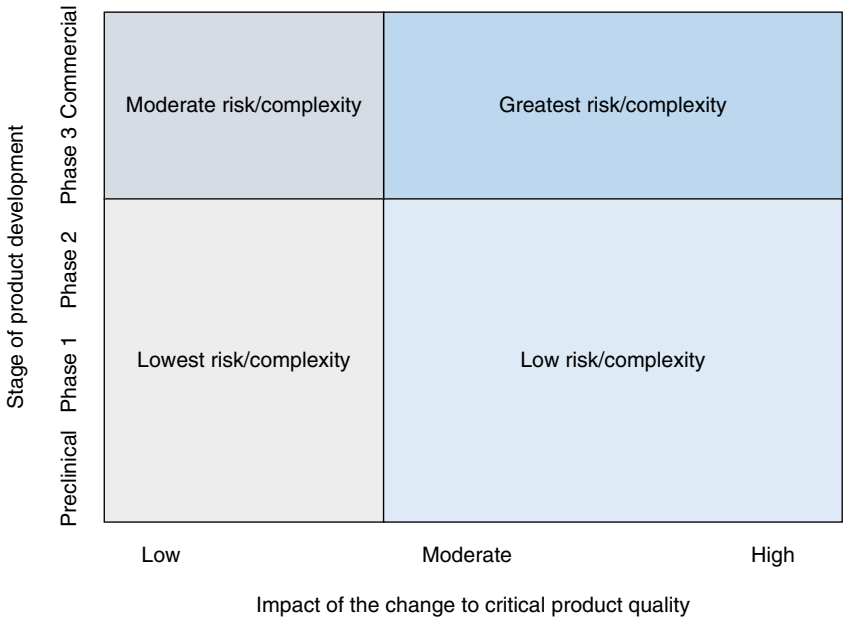


Figure 15.3 Factors influencing comparability complexity: stage of product development and impact of the change to critical product quality.

product and process knowledge are gained, and appropriate and specific analytical methodologies to characterize the product become available. From a risk management perspective, changes made later in development present a higher risk than those implemented earlier. Consequently, regulatory agencies will expect a larger body of data from such comparability assessments and also apply more stringent comparability criteria for manufacturing process changes made during the later stages of development.

Analytical methods used for a comparability exercise during development should be appropriate and suitable for their intended purpose. While the methods may not have been fully validated, they should be scientifically sound and provide reliable and reproducible results. Due to the limitations of the analytical tools in early development, physicochemical and biological tests alone may be considered inadequate in determining comparability. Therefore, data from bridging nonclinical and/or clinical studies may be needed.

15.4.10 Nonclinical and Clinical Considerations

Product comparability can be based solely on quality considerations if assurance of comparability can be provided through analytical studies. Additional evidence from nonclinical and clinical studies is appropriate when quality data are insufficient to establish comparability. The need for nonclinical and clinical studies to assess comparability is based on the following considerations:

- Quality considerations: The extent of differences between pre- and post-change products and whether new impurities or product-related variants are formed may need to be qualified in, for example, toxicology, animal PK, or even human bioequivalence studies. Additionally, the capabilities and limitations of tests used in the comparability studies may warrant nonclinical and/or clinical evaluation of the product.
- Knowledge of the product:
  - Physicochemical and *in vitro* biological assays may not be able to detect differences in the structure and/or function due to product complexity including heterogeneity and higher-order structure.
  - The relationship between quality attributes and safety/efficacy may not be fully understood (structure–activity relationships).
  - Relationship between therapeutic protein and endogenous proteins and the consequences for immunogenicity.
  - Modes of action (unknown vs. known, single vs. multiple active sites).
- Existing nonclinical and clinical data:
  - Therapeutic indications/target patient groups—Impact of product differences can vary between patient groups (e.g., risk of immunogenicity). Therefore, the analysis of risk should be done separately for each indication.
  - Posology (e.g., dosing regimen, route of administration)—Risk of immunogenicity can be higher with chronic administration as compared with

short-term administration. Similarly, SC administration of the drug may induce immunogenicity more often than intravenous administration.
- Therapeutic window/dose-response curve—Impact of certain changes could be different for products with wide therapeutic windows compared with those that have a narrow therapeutic window. The safety or efficacy of products with a steep or a bell-shaped dose-response curve can be affected by minor changes in PK or receptor-binding.
- Previous experience (e.g., immunogenicity, safety)—Knowledge gained from the original product or from other products in the same product class can be valuable in assessing the risk of adverse events (e.g., immunogenicity risk).
- PK/PD relation, distribution, and clearance.

Types of studies—Nonclinical and clinical studies enable comparison of the pre- and post-change products. These may include PK studies, PD studies, PK/PD studies, clinical efficacy studies, specific safety studies, immunogenicity assessments, and pharmacovigilance studies. Where appropriate, these studies should be conducted as direct comparative studies (i.e., pre-change vs. post-change).

15.5 Conclusions

Manufacturing process changes are part of the product development continuum and are inevitable over the life cycle of a biological product. While a process change may seem minor in nature, the change may have a great impact on the safety and efficacy profile of the therapeutic protein. Manufacturers must therefore have a good understanding of the product and its manufacturing process, including knowledge of the critical product attributes and the relationship between the product's structural characteristics and function. Molecular characteristics that are inherent to the molecule, including heterogeneity and higher-order structure, should be considered in the design of the comparability study. The authors have provided examples and case studies to further illustrate the practical application of the ICH Q5E guideline to real-life biological product manufacture. The availability of relevant data from early development, validation studies, stability studies, and clinical data dictates the nature and extent of testing required to characterize a biological product. The data expectations for a comparability study increase as the product moves from early development to late-stage development through marketing authorization. Because each product and change are unique, comparability assessments have to take into account the body of knowledge that the manufacturer possesses for that product before designing a comparability study.

References

1 United States, Food and Drug Administration (US FDA) 1996. FDA guidance concerning demonstration of comparability of human biological products, including therapeutic biotechnology-derived products.
2 United States, Food and Drug Administration (US FDA) 1997. Guidance for Industry on changes to an approved application for biological products and specified biotechnology and synthetic biological products.
3 European Medicines Agency 2003. Guideline on comparability of medicinal products containing biotechnology-derived proteins as active substance: Quality issues. EMEA/CPMP/BWP/3207/00/Rev1.
4 European Medicines Agency 2003. Guideline on comparability of medicinal products containing biotechnology-derived proteins as active substance: Non-clinical and clinical issues. EMEA/CPMP/3097/02/Final.
5 International Conference on Harmonisation (ICH) 2004. Q5E. Comparability of biotechnological/biological products subject to changes in their manufacturing process.
6 Lewis RM, Cosenza ME 2010. Summary of DIA workshop: Comparability challenges: Regulatory and scientific issues in the assessment of biopharmaceuticals. *Ther Innov Regul Sci* 44:485–504.
7 Hendrickson TL, Imperiali B 1995. Metal ion dependence of oligosaccharyl transferase: Implications for catalysis. *Biochemistry* 34:9444–9450.
8 Pacis E, Yu M, Autsen J, Bayer R, Li F 2011. Effects of cell culture conditions on antibody N-linked glycosylation—What affects high mannose 5 glycoform. *Biotechnol Bioeng* 108:2348–2358.
9 Bower KM, Germansderfer A 2010. Determination of comparability criteria: Statistical strategies to address the challenges imposed by limited data. *Genet Eng Biotechnol News* 30.
10 Cowley D, Kendrick BS, Sidor L, Burdick R 2011. Demonstrating comparability of stability profiles using statistical equivalence testing. *BioPharm Int* 24:36–42.
11 Chirino AJ, Mire-Sluis A 2004. Characterizing biological products and assessing comparability following manufacturing changes. *Nat Biotechnol* 22:1383–1391.
12 Parenteral Drug Association (PDA) 2015. Analytical method development and qualification for biotechnology products. Parenteral Drug Association (PDA) Technical Report No. 57-2.
13 International Conference on Harmonisation (ICH) 1994. Q2(R1). Validation of analytical procedures: Text and methodology.
14 International Conference on Harmonisation (ICH) 1995. Q5C. Quality of biotechnological products: Stability testing of biotechnological/biological products.

15 International Conference on Harmonisation (ICH) 1999. Q6B. Specifications: Test procedures and acceptance criteria for biotechnological/biological products.
16 Van Den Hamer CJ, Morell AG, Scheinberg IH, Hickman J, Ashwell G 1970. Physical and chemical studies on ceruloplasmin. IX. The role of galactosyl residues in the clearance of ceruloplasmin from the circulation. *J Biol Chem* 245:4397–4402
17 Niazi SK 2004. *Handbook of Pharmaceutical Manufacturing Formulations: Sterile Products*. CRC Press, Boca Raton, London, New York, p 98.

16

ICH Q6A Specifications

Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances

David Elder

David P Elder Consultancy, Hertford, UK

16.1 Introduction

A specification is defined by ICH Q6A [1] as "a list of tests, references to analytical procedures, and appropriate acceptance criteria, which are numerical limits, ranges, or other criteria for the tests described." It establishes a predefined set of criteria (which have been either internally approved or approved by the appropriate regulatory agency) to which an API or a drug product should comply in order to be considered appropriate for its anticipated use. Specifications are therefore critical quality standards (CQSs) linked to overall product performance, which are proposed and endorsed by the applicant and reviewed and approved by the appropriate regulatory or pharmacopoeial authorities. Interestingly, it is by no means certain that acceptance by one agency (e.g., FDA) necessarily implies/leads to approval by others (e.g., EMA, PMDA), and therefore the concept of a universal specification, for an API/drug product, is often difficult to achieve, despite this being expressly stated as a key objective of this guideline [1]. This is particularly apparent in relation to pharmacopoeial tests (see Chapter 9 for a full perspective on the challenges faced).

Importantly, specifications are chosen to confirm the underlying quality of the API and drug product rather than establishing full characterization of those critical processing parameters (CPPs) and critical quality attributes (CQAs), which are generated as part of a Quality by Design (QbD [2]) submission. As such, the specifications focuses on those CQAs ascertained to be important in establishing and maintaining the safety and efficacy of the API and drug product.

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16.2 Scope of the ICH Q6A

16.2.1 Overview of ICH Q6A

The intrinsic quality of both API and drug products is defined by their design phase (quality target product profile (QTPP)), knowledge gained during development (QbD [2]), optimization of the supporting manufacturing processes (including appropriate in-process controls and validation), supporting GMP infrastructure, and supporting specifications (in-process, at release, and at the end of shelf life). Note that some authorities only recognize the end of shelf-life specifications (e.g. FDA, PMDA). See Section 16.3 for a fuller discussion.

The ICH Q6A [1] guideline indicates that specifications form part of the overarching control systems (or control strategy) that help to assure the quality of the API and drug product at release and at the end of shelf life. Although specifications are an important part of this process, they should not be considered in isolation of these other considerations, which are essential to ensure consistent production of API and drug products of high quality. Other aspects of this control strategy include (but are not limited to) comprehensive API/product characterization (design space, CQAs, CPPs, proven acceptable range (PAR), and acceptable ranges) and the understanding obtained during development together with strict adherence to good manufacturing practice (GMP) and its underpinning requirements, for example, appropriate facilities, validated testing and manufacturing processes, in-process and raw material testing, stability testing of API and drug product, etc.

The specification acceptance criteria are typically comprised of numerical limits (often one sided, i.e., NMT 0.2%), ranges (often asymmetrical around the median, i.e., 98.0–101.5%), or other suitable criteria for the tests described (i.e., sterile). Thus, "conformance to specification" means that the API and/or the drug product, when tested according to the specification, will comply with the predefined acceptance requirements. Thus, for instance, an API that claims to be USP grade must comply with all of the tests in the USP monograph for that API. Although specifications define CQSs, not all of the tests outlined will be CQAs, but all tests will be quality attributes; similarly, not all CQAs will necessarily be specification tests. For example, a mutagenic impurity can be controlled on the specification (option 1, ICH M7 [3]), but equally it can be controlled using other agreed approaches (options 2–4, ICH M7).

It is possible that specifications may also define additional in-process tests, periodic (skip) tests, or other tests, which are not always routinely performed on every batch (see Section 16.3). In those cases, the manufacturer is required to specify those tests that are routinely performed to release all batches, together with a commentary and appropriate justification of the full-testing frequency, for example, twice a year. Where a full testing is performed, the API

and/or drug product are required to meet the acceptance criteria for all tests, including nonroutine tests.

16.2.2 Specification Justification

The specifications that are ultimately submitted in the New Drug Application (NDA)/Marketing Authorisation Approval (MAA) applications are the result of an ongoing dialogue with the relevant regulatory authorities, that is, multiple Investigational New Drug (IND)/Investigational Medicinal Product Dossier (IND/IMPD) submissions and related meetings, over the course of the clinical development program. However, at the time of submission, the proposed specification will still require a full supporting rationale. This justification should be provided for every test and every proposed acceptance criterion. The justification should encompass the historical regulatory dialogue, relevant development data, relevant pharmacopoeial standards, and relevant test data for API or drug products used in pivotal nonclinical safety and pivotal clinical studies and all results from available accelerated and long-term stability studies. In addition, a "reasonable" range of anticipated analytical and manufacturing variability should be considered. This review should also consider the analytical target profile (ATP) of the supporting method (see ICH Q2(R1) [4]). Although the justification can consider theoretical variability for a given process or acceptance criteria, the actual data forms the main basis for any approval. This is the only mention of variability given in the guidance document, which is a major deficiency given that many/most specifications do not accurately reflect the likely variability, or are artificially constrained and are not likely to reflect typical variability, that is, tightening of specification based solely on clinical batch performance, without considering the performance of all batches that have been used during development, including stability considerations. This will be discussed in greater depth later in this chapter (see Section 16.5).

Data from stability batches, particularly scale-up and/or validation batches (with a particular focus on the primary stability batches), should be assessed when defining and justifying specifications. If more than one manufacturing site is proposed, then it may be necessary to assess data from these additional sites when establishing any specification acceptance criteria, especially if there are limited data derived from the manufacture of the API or drug product at any additional site(s). However, if data from only one manufacturing site is used in setting specification acceptance criteria, any product subsequently manufactured at another site(s) should still comply with those release criteria.

16.2.3 Applicability of ICH Q6A during Development

The ICH Q6A guidelines were intended to be formally applicable to the marketing approval of new APIs/drug products, including fixed-dose combination (FDC) products. Consequently, they were never intended to be applied to

either APIs or drug products during the clinical research phases of drug development. During development, knowledge of CQAs may be limited, as significant changes in both synthesis/process (API) and formulation/process (drug product) would be anticipated. ICH M3(R2) [5] provides some useful insight for qualifying impurities during the development phase: "The approaches for qualifying impurities and degradants are outlined in ICH Q3A and Q3B. If specific studies are warranted to qualify an impurity or degradant, generally these studies are not warranted before Phase III, unless there are changes that result in a significant new impurity profile e.g., a new synthetic pathway, a new degradant formed by interactions between the components of the formulation." As the API and drug product evolve toward the final commercial processes, CQAs will be identified, trended, and specified. There may be a requirement to manufacture some batches at the proposed commercial scale in order to fully specify CQA limits of acceptable ranges.

However, a recent industry publication cautioned against the default perception that data generated on pilot batches was not predictive of full-scale production batches: "The outcome of such a science- and risk-based assessment should focus on the true risk of scale-dependency rather than a broad expectation for verification across a broad design space on the commercial scale during development" [6].

Despite these caveats, the reality is that ICH Q6A guidance is widely used during development by both industry and regulators (often inappropriately so, i.e., requests for full details of impurities to be included on early-phase clinical specifications, in some instances combined with requests to clearly identify acceptance criteria), and it is difficult to conceive of compiling any early-phase regulatory submission, for example, IND and IMPD, without utilizing some aspects of the ICH Q6A guidance. Great care is needed to ensure that specifications are not fixed at an early stage based on limited data as this may artificially constrain the formal specification at the time of marketing, especially if there is an intention to remove tests specified in early versions. For instance, it would be inappropriate to add an impurity generated by an early stage API process on the registration specification, if it can be clearly demonstrated that this impurity cannot form using the final registered process.

16.2.4 Specification Scope (ICH Q6A vs. ICH Q6B)

Although the ICH Q6A [1] guideline could be useful for synthetic and semi-synthetic antibiotics, peptides of low molecular weight or oligonucleotides, it certainly does not adequately describe specifications of higher molecular weight peptides, polypeptides, and biotechnological/biological products. This is addressed instead in the complementary ICH Q6B [7] guidance that specifically addresses the establishment of specifications, tests, and procedures for

biotechnological/biological products. Radiopharmaceuticals, herbal products, fermentation products, oligonucleotides, and "crude" products of animal or plant derivation are similarly not covered, but the guidance is often used to facilitate specification setting.

16.2.5 Specification Format

In terms of its format, the ICH Q6A [1] guidance is subdivided into universal tests applicable to all APIs and drug products, that is, description, identity, assay, and impurities, as well as those specific tests that are applicable on a case-by-case basis. However, this guidance should not be considered to be all-embracing as novel analytical technologies, and upgrades to existing technology, are continually being developed. Therefore, such innovative technologies should be used when appropriate and justified.

Solid oral dosage forms, that is, tablets, capsules, liquid oral dosage forms, that is, solutions, suspensions, emulsions, and parenterals, both small and large volume, are the primary focus of this guidance. This guidance may however be applicable to other medicinal products. The application of the guideline concepts to other dosage forms, for example, to inhalation dosage forms (powders, solutions, etc.), to topical formulations (creams, ointments, gels, etc.), and to transdermal systems, is therefore encouraged. However, despite numerous discussions at ICH committee meetings, the original guidance has never been updated and does not feature in the future program of work for updated/new ICH guidance documents. Interestingly, the USP provides a broader perspective on requirements for other product types not covered by ICH Q6A, in USP <1151> Pharmaceutical Dosage Forms [8].

16.2.6 Specifications and Change Control

As the specification is a critical part of the control of the API/drug product and is agreed through the submission process with regulatory authorities, any post-approval modifications to the specification will require approval by the appropriate regulatory authority. Dependent on the nature of the change, this could be a major (prior approval) or minor regulatory submission.

16.3 ICH Q6A General Concepts

The following general concepts are critically important in the development of harmonized specifications, with the caveat that true harmonization may not be fully attainable (see ICH Q4 [9]). The guideline provides a brief synopsis of each general concept and of the situations where they may be pertinent. These have been summarized in Table 16.1 and are illustrated in Figure 16.1.

Table 16.1 ICH Q6A general concepts.

General concepts	Overview
Periodic or skip testing (or periodic verification testing)	Performing specified tests, for example, residual solvents, impurity, or microbiological testing at release on preselected batches and/or at predetermined intervals, rather than on every batch. However, all batches not being tested still must meet all acceptance criteria established for that product. It requires pre-justification by the regulatory authority prior to initiation. If data subsequently demonstrate a need to restore routine testing, then batch release testing should be reinstated A major challenge in the practical implementation of such testing regimes is how many batches (skip) and/or how long between testing (periodic)? There is no clear guidance provided on this point either in ICH Q6A or any other formal guideline, as such the applicant should initiate regulatory dialogue to address these considerations
Release vs. shelf-life acceptance criteria	Establishing tighter acceptance criteria for a drug product specification at release compared to at the end of shelf-life, for example, assay or related substances specifications. In the EU this is a legal requirement. In contrast, in Japan and the United States, the acceptance criteria remain unchanged from release to the end of the products' shelf life. Applicants may decide to have tighter "in-house" specifications at release—providing assurance that the product will continue to remain within the defined acceptance criterion throughout its shelf life
In-process tests	Performed during the manufacture of either the API or drug product, rather than as part of the formal release specification. Typically, these tests are not included in the release specification, for example, individual tablet weights, hardness, and friability. If the acceptance criteria are either identical, or indeed tighter, than the release requirements, for example, the pH of a solution or suspension, this can be used in lieu of testing (when the test is included in the specification)
Design and development considerations	1) It may be possible to recommend exclusion or replacement of certain tests based on underpinning development knowledge, for example, leachables from drug product containers, where either no extractables/leachables are found in the drug product or the levels meet accepted standards 2) API or drug product mutagenic impurities (MIs), where it has been consistently shown that either no MIs are found in the API or drug product or the levels meet accepted standards defined in ICH M7 [3] (i.e., options 2–4) 3) Particle size testing, performed either as an in-process test or as a release test, depending on its importance to drug product performance 4) Dissolution testing for immediate release solid oral dosage forms for BCS class I drugs may be replaced by disintegration testing, if these products have historically been demonstrated to have rapid drug release characteristics 5) Microbiological testing for API and solid dosage forms, which have been demonstrated not to support microbial growth or microbial viability (often based on water activity arguments), for example, USP <1112> Water Activity and a full microbiological risk assessment, for example, USP <1115> Bioburden Control of Non-sterile Drug Substances and Products, Ph. Eur. 5.1.4., Microbiological Quality of Non-Sterile Pharmaceutical Preparations and Substances for Pharmaceutical Use

Table 16.1 (Continued)

General concepts	Overview
Limited data available at filing	The initially approved specification may be reassessed as more information becomes available, with the goal of potentially modifying the registered specification. In theory, this could involve loosening, as well as tightening, of specification acceptance criteria, although the former approach can be very challenging
	Setting a specification based on limited data is especially challenging in circumstances where the product is fast tracked, often skipping clinical trial phases, for example, oncology products. In such instances it is important to ensure that the specification isn't artificially tightened, resulting in arbitrary failures that may not actually impact on product performance. It will be interesting to see how the proposed regulatory guidance for fast-tracking drug product development [9, 10] will address this issue
Parametric release	Can be performed either in-line or at-line and can be used as an alternative approach to routine release testing for API or drug products. An example of such an approach is sterility testing for parenteral drug products where the final release is based on acceptable output from ongoing assessments of critical process parameters (CPP), for example, temperature, pressure, and time, which are continuously monitored as part of the sterilization procedure. These CPPs are more predictive of sterility assurance than standard end-product sterility testing. Importantly, when parametric release is carried out, the attribute(s), which are indirectly controlled (e.g., sterility), are still included on the specification and routinely performed as part of batch release. Guidance on parametric release [11] and real-time release testing (RTRT) is available [12]
Alternative procedures	These alternative procedures control CQAs of the API or drug product to a comparable or indeed superior extent than the official procedure, for example, fast micro-methods [15] versus microbial tests or NIR for assay versus HPLC. However, in both examples, the official procedure is still used to demonstrate compliance with the specifications' acceptance criteria throughout the shelf life of the product
Pharmacopoeial tests and acceptance criteria	These tests should always be used as API specification tests where possible, for example, water, heavy metals, sulfated ash, etc., or new drug product tests, for example, disintegration, dissolution, uniformity of content, etc. However, if differences in a defined pharmacopoeial procedures and/or acceptance criteria exist between the ICH regions, then a harmonized specification is only possible if these tests are acceptable to regulatory authorities in all of the ICH regions (see ICH Q4B [8]). All three pharmacopoeias identify in their monographs when tests are considered comparable and interchangeable, and a suitable reference to the harmonized test and acceptance criteria is considered appropriate for any specification across the three ICH regions

(Continued)

Table 16.1 (Continued)

General concepts	Overview
New analytical approaches	Such novel analytical methodologies should be used when they are considered to offer additional assurance of quality, safety, or efficacy or when otherwise suitably justified, for example, addition of orthogonal purity tests when the specificity of any one test cannot be guaranteed, for example, protein, oligonucleotides, oligosaccharides, and drug product assay testing. For instance, rapid microbiology tests offer significant advantages versus the standard pharmacopoeial tests [13]
API versus drug product specifications	It should not be necessary (nor required) to test the drug product for quality attributes uniquely associated with the API. For example, it is not necessary to test the drug product for synthetic impurities controlled in the API specification, as long they are not also degradation products. Also, if it can be demonstrated that racemization of the API cannot occur during drug product manufacture or storage, then there is no requirement to perform either chiral purity or chiral identity tests on the drug product

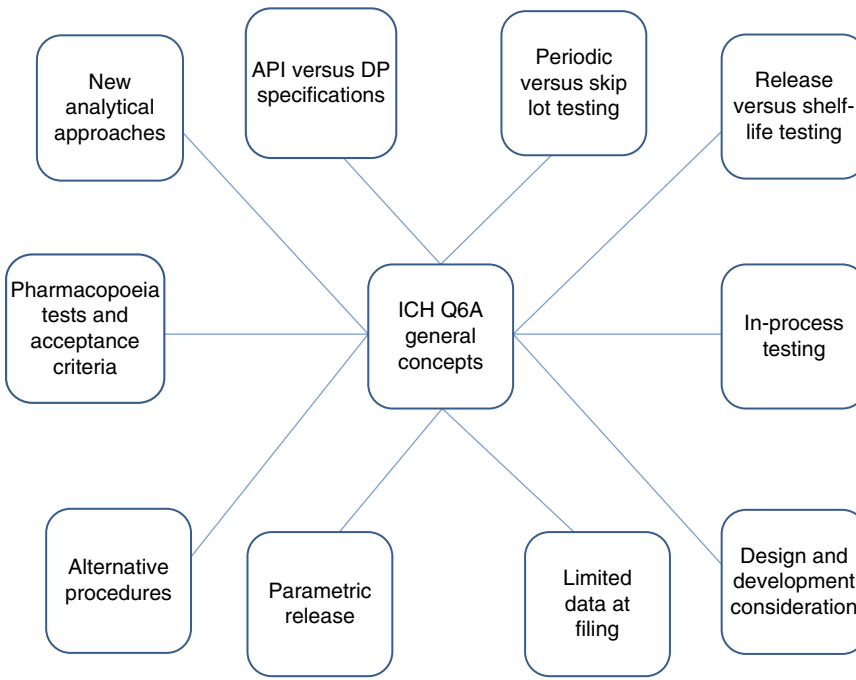


Figure 16.1 Schematic showing ICH Q6A general concepts.

16.4 ICH Q6A Guidelines

16.4.1 Universal Tests/Criteria

Description, identity, assay, and impurities (or degradants for drug product impurities) are considered generally applicable to all new APIs and drug products.

16.4.1.1 Description

Description is a qualitative statement about the physical state, for example, solid, liquid, color, and general appearance, for example, crystalline, noncrystalline, etc., of the API. For the drug product the description will describe the appearance (including color and any printed or embossed logos or features, such as a tablet break line or banding of a capsule or unique identifying code) and physical dimensions of solid oral dosage forms, for example, tablets and capsules. For liquid presentations, description will also cover the container closure, for example, 150 ml amber glass bottle with a child-resistant cap or 1 ml blow fill sealed ampoule (or nebule) as well as the physical state (solid, liquid, color, and general appearance, e.g., clear colorless, free from visible particulate matter). Appearance can sometimes erroneously be used in lieu of description, but this is more correctly viewed as a subset of description.

Generally, other organoleptic considerations, such as taste or smell, are not included on the specification to deter analysts from tasting or smelling potent pharmacologically active substances. However, if there is a pronounced odor that can be discerned without actively smelling the API, it should be commented upon, for example, benzaldehyde has a characteristic almond-like odor. In contrast, for liquid drug products, a flavorant may well have been added as part of the design intent (QTPP) in which case this should be described, that is, orange viscous liquid with pronounced citrus flavor. If any of these attributes change during storage, or on stability, this change should be assessed and appropriate action should be taken. For instance, if significant changes occur during photostability testing (see ICH Q1B [14]), then the description of the API/drug product may be supplemented by a quantitative measurement, for example, using Munsell color notation or tristimulus colorimetry (TSC). Recently, Hetrick et al. [15] outlined the role that TSC can play in a QbD development strategy. They showed that TSC can remove some of the subjectivity that plagues visual assessment of color and particularly color changes, which can result in different assessments (operator to operator bias).

16.4.1.2 Identification

Identification tests should ideally be able to differentiate between the API and closely related structures, which are *likely* to be present as impurities. Identification tests should be specific (or selective) for the API, for example,

infrared spectroscopy (IR) and nuclear magnetic resonance (NMR) spectroscopy. Identification using nonspecific approaches is generally regarded as being inappropriate. If the API is a salt or a cocrystal, then identification testing should be specific for the individual counterions/conformers, for example, ion chromatography, IEX-HPLC (Ion Exchange-HPLC), or ICP-MS (Inductive Coupled Plasma-MS) could be used for Na, K, and other counterions.

For an enantiomerically pure API, the identity test(s) needs to be capable of discriminating both enantiomers, as well as the racemic mixture. For a racemic API, there are generally two scenarios where a stereospecific identity test is appropriate for release testing: (i) where there is a high probability that the single enantiomer might be substituted for the racemate or (ii) when there are data showing that preferential crystallization can yield unintended formation of a non-racemic mixture. ICH Q6A [1] decision tree 5 directs as to when or indeed if chiral identity tests, impurity tests, and assays are needed for APIs and drug products.

Similarly, identification tests for drug products should be specific (or selective). However, a nonspecific identity test, for example, ultraviolet (UV) spectroscopy could be used in combination with a specific identity test, for example, HPLC. There are no requirements to repeat identity tests, where the identity of an attribute has been unequivocally demonstrated in the API, for example, identity of counterion, chiral purity, etc. (see Table 16.1: API vs. Drug Product).

16.4.1.3 Assay

Assay tests require a specific stability-indicating method to be used to determine API content in both the drug substance and the corresponding drug product. It is possible to employ the same procedure, for example, HPLC, for both assay of the API and the impurities, although the same operating conditions are not always employed nor is the same sensitivity necessarily expected or achievable. In addition, some type of selective extraction or matrix deactivation may be required for the impurity assay, due to sensitivity considerations (see Chapter 5: ICH Q2(R2)). Similarly, the same procedure, for example, HPLC, is often used for assay of both API and drug product, but again, the same operating conditions are not always employed. Output from content uniformity testing for drug product can also be utilized for assay of drug product, providing that the methodologies utilized for former are also appropriate for assay determination, that is, specific. In those cases where a nonspecific assay can be justified, for example, superior precision of nonaqueous titration method for API assay, other orthogonal analytical methods need to be utilized to achieve acceptable specificity, for example, a suitable impurities assay. A specific method should always be used for drug products when excipient interference is likely for nonspecific assays.

An enantioselective assay should form part of the API specification. It is considered adequate for this to be achieved either using a chiral assay method or by the use of an achiral assay together with appropriate orthogonal approaches for controlling any enantiomeric impurities. An achiral assay may be satisfactory for the drug product specification (see Table 16.1: API vs. Drug Product). ICH Q6A decision tree 5 shows when or indeed if chiral identity tests impurity tests and assays (suitably stability indicating), are needed for APIs and drug products.

16.4.1.4 Impurities

Impurities tests including related impurities and/or degradation products, residual solvents (see ICH Q3C(R5) [16]), residual metals (see ICH Q3D [17]), and mutagenic impurities (see ICH M7 [3]). Those impurities arising during the synthetic manufacturing process for the API and those arising from degradation of the API/drug product should be monitored on the API and drug product specifications, respectively. For drug products, acceptance limits are ascertained for individual degradation products (which include both identified and unidentified degradation products, as appropriate), as well as the total degradation products. However, in those cases when an impurity from the API synthetic route is also a degradation product, these need to be monitored and controlled, and they need to be included in the total degradation product limits (see Table 16.1: API vs. Drug Product). ICH Q6A [1] decision tree 2 addresses the determination of meaningful limits for degradation products from the available development data.

Where an API is primarily a single enantiomer, the reverse or "unwanted" enantiomer is exempted from the qualification and identification thresholds given in the ICH Q3A(R2) [18]/Q3B(R2) [19] due to the practical analytical challenges associated with quantification at these very low levels. However, this "unwanted" enantiomeric impurity still needs to be controlled and specified using the principles established within those guidelines (Q3A(R2) [18]/Q3B(R2) [19]). In addition, appropriate testing of a starting material or intermediate, with suitable justification, could also provide greater assurance of control (see Table 16.1: API vs. Drug Product). ICH Q6A decision tree 5 directs as to when or indeed if chiral identity tests, impurity tests, and assays are needed for APIs and drug products.

16.4.2 Specific Tests/Criteria

The following additional tests may be required for either API (see Table 16.2) or drug product (see Tables 16.3 and 16.4) specifications. Tests on CQAs will likely be included in the API and/or drug product specifications, but there are scenarios where they may be excluded (see options 2–4, ICH M7 [3]). However, tests in addition to those highlighted in the following text may be required in specific situations or as new information is obtained.

16.4.2.1 API Specific Tests

Table 16.2 ICH Q6A additional specific tests for APIs.

Additional test	Commentary
Physicochemical properties	The tests are defined by the physical nature of the API and by its planned use. These tests reflect intrinsic properties of the compound, such as melting point/range, refractive index, etc. The methods used are well defined in the relevant pharmacopoeias, for example, melting point (USP <741>, Ph. Eur. 2.2.14), refractive index (USP <831>, Ph. Eur. 2.2.6), etc.
Particle size and related characteristics	For APIs with poor solubility characteristics, for example, biopharmaceutics classification system [20], that is, BCS class II (poor solubility/good permeability)/IV (poor solubility/poor permeability) compounds, intended for use in either solid or suspension dosage forms, then particle size can affect dissolution rates, bioavailability, and in some cases stability. Thus, particle size distribution testing may be required and relevant acceptance criteria assessed. Particle size can be assessed using different analytical techniques, for example, particle size distribution estimation by analytical sieving (USP <786>, Ph. Eur. 2.9.38), light diffraction measurements (USP <429>, Ph. Eur. 2.9.31). Other complimentary techniques are optical microscopy (USP <776>, Ph. Eur., 2.9.37) and specific surface area by gas adsorption (USP <846>, Ph. Eur., 2.9.26). ICH Q6A [1] decision tree 3 gives additional guidance on when particle size testing should be evaluated
Polymorphic form	For those compounds with poor solubility characteristics, for example, BCS (Biopharmaceutics Classification System) [20] class II/IV, where polymorphic (also hydrate/solvate, cocrystal or salt) differences can affect quality, processability, or the *in vivo* performance of the drug product, then the appropriate solid state should be specified and characterized. Appropriate tests include optical microscopy, thermal methods, for example, melting point, hot-stage microscopy, DSC (Differential Scanning Calorimetry), TGA, and DTA (Differential Thermal Analysis), X-ray techniques, spectroscopic methods, for example, IR, Raman, and solid-state NMR, etc. Some polymorphic forms can have very different habits (see Figure 16.2), and the influence on processability is very clear. ICH Q6A decision trees 4(1)–4(3) offer supplementary guidance on when, and how, polymorphic forms need to be monitored and controlled. Decision trees 4(1) and 4(2) evaluate whether polymorphism is shown by the API and whether these different polymorphic forms can subsequently affect drug product performance. Decision tree 4(3) is only applied when polymorphism has been shown for the API and shown to affect these critical properties

Table 16.2 (Continued)

Additional test	Commentary
	It is often very challenging to measure polymorphic changes in drug products due to sensitivity considerations. However, it is a common request from NDA/MAA reviewers. Alternatively, other tests, for example, dissolution, can generally be used in lieu of these specific polymorphic tests to routinely monitor product performance. The guidance indicates that polymorph content would only be used as a test of last resort (see decision tree 4(3))
Water content	This specification test is essential in those cases where the API is known to be either hygroscopic or susceptible to hydrolysis or when the API is known to be a stoichiometric hydrate (or can form stoichiometric hydrates). Specification limits can be justified with information on the effects of hydration or moisture adsorption/absorption. In certain cases, a loss on drying (LOD) procedure may be considered adequate (USP <731>, Ph. Eur. 2.2.32.); however, a specific procedure for water, for example, Karl Fischer titration, is preferred (USP <921>, Ph. Eur.2.8.)
Inorganic impurities	The requirements for inclusion of limit tests and acceptance criteria for residual metal impurities (e.g., catalysts) are discussed in a separate chapter (ICH Q3D [19])
Mutagenic impurities	The requirements for inclusion of either in-process tests or limit tests and acceptance criteria for mutagenic impurities are discussed in a separate chapter (ICH M7 [3])
Microbial limits	Where microbiological quality of an API is important (manufacture of sterile products) or where the levels of "available" water are high, giving rise to concerns about microbiological spoilage, this test should be specified. This is done using the total count of aerobic microorganisms, the total count of yeasts and moulds, and the absence of specific pathogenic bacteria (e.g., *Staphylococcus aureus*, *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa*). In addition, sterility and endotoxin testing may also be appropriate for APIs manufactured as sterile (e.g., sterile crystallized antibiotics) or APIs used to formulate sterile drug products
	Microbial limits should be tested according to the appropriate pharmacopoeial methods (USP <61>, USP <62>, USP <1116> Bioburden Control of Non-sterile Drug Substances and Products, Ph. Eur. 5.1.4., Microbiological Quality of Non-Sterile Pharmaceutical Preparations and Substances for Pharmaceutical Use). The type of microbial test(s) and the appropriate acceptance criteria are based on the nature of the API, method of manufacture, and intended use of the drug product. ICH Q6A decision tree 6 provides supplementary guidance on what microbial limits are applicable

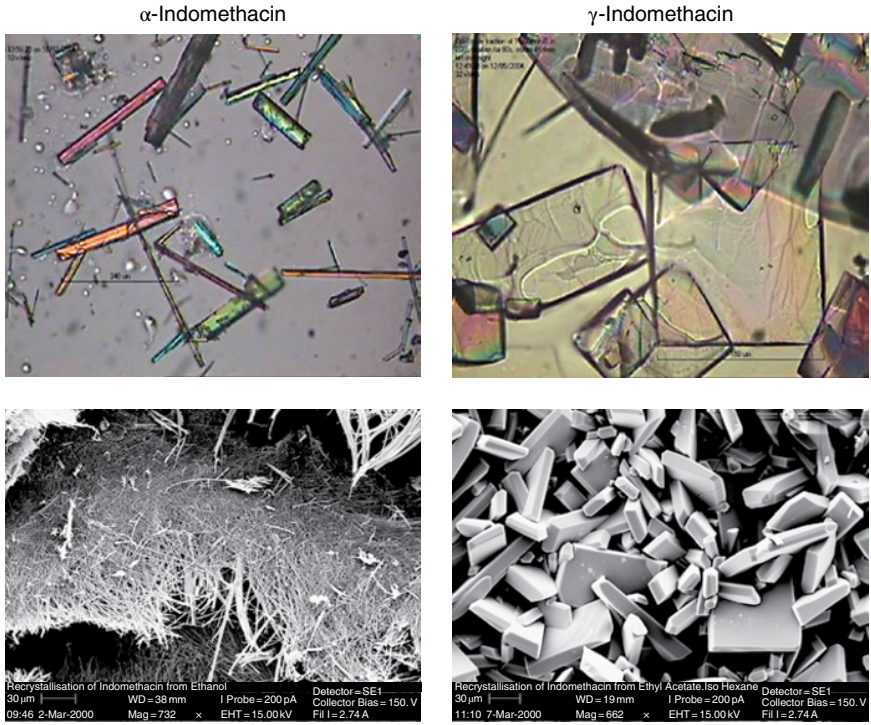

α-Indomethacin
γ-Indomethacin
Recrystallisation of Indomethacin from Ethanol
30 µm WD = 38 mm I Probe = 200 pA Detector = SE1
09:46 2-Mar-2000 Mag = 732 × EHT = 15.00 kV Fil I = 2.74 A
Recrystallisation of Indomethacin from Ethyl Acetate.Iso Hexane
30 µm WD = 19 mm I Probe = 200 pA Detector = SE1
11:10 7-Mar-2000 Mag = 662 × EHT = 15.00 kV Fil I = 2.74 A

Figure 16.2 Scanning electron microscopy pictures of polymorphs of a drug substance (showing pronounced differences in habit). Courtesy of AstraZeneca.

Table 16.3 Additional specific tests for drug products (solid oral dosage forms).

Drug product additional tests	Commentary
Disintegration	Specifications for solid oral dosage forms typically include a test to measure the *in vitro* release of drug substance from the drug product, that is, dissolution. However, in the case of rapidly dissolving dosage forms, this test can be replaced with disintegration testing (see decision tree 7(1)). A recent commentary provides some additional guidance when considering disintegration as a dissolution surrogate [21].The test is fully described in all of the pharmacopoeias (e.g., USP <701>, Ph. Eur. 2.9.1) and in Al-Gousous and Langguth [21]. Although the test is harmonized between the different pharmacopoeias, there are some non-harmonized local requirements

Table 16.3 (Continued)

Drug product additional tests	Commentary
Dissolution (immediate release)	Single-point measurements, for example, 30 min, are normally considered to be suitable for immediate-release dosage forms, particularly for BCS class 1 (good solubility, good permeability) drug products [22]. However, for rapidly dissolving dosage forms or BCS class 3 (good solubility, poor permeability), single-point measurements at 15 min may be more appropriate [24]. Multipoint measurements, for example, 15, 30, and 45 min, are often made when assessing the long-term stability of an immediate-release drug product as this facilitates comparison of dissolution profiles as a function of storage time and storage conditions, including demonstrating equivalence of halved-tablets, where the product has a functional break line. Where changes in the *in vitro* dissolution rate have been demonstrated to significantly affect bioavailability, it is important to develop test conditions that can distinguish batches with undesirable bioavailability. If changes in formulation or processing conditions can significantly affect dissolution and these changes are not controlled by another specification test, it may also be desirable to utilize dissolution test conditions that can differentiate these changes, that is, acceptance criteria should be set to reject batches with unacceptable bioavailability (see decision tree 7(2)). Otherwise, both dissolution test conditions and acceptance criteria should be agreed, which pass clinically acceptable batches (see decision tree 7(2)). Unfortunately, method capability is rarely discussed as part of these discussions (this is further discussed in section "Process Capability and Specification Setting"). Although the test is harmonized between the different pharmacopoeias, there are some non-harmonized local requirements
Dissolution (modified release)	For modified-release (or controlled-release or extended-release) dosage forms, appropriate dissolution test conditions and time points should be established. Multiple time points are required for these dosage forms, for example, early, middle, and late time points, to fully characterize the release profile. Delayed-release dosage forms, for example, enteric-coated drug products, typically require two-stage dissolution testing (using dissimilar media with different pHs). Relevant additional guidance is also provided by FDA [23] and EMA [24]. For these drug products it is imperative to consider both the patient populations and likely drug coadministration, for example, patients taking PPIs (elevated gastric pH), achlorhydric patients (elevated gastric pH), and elderly patients (different GI transit times, elevated gastric pH, co-mediations, e.g., PPIs), when designing both the tests and acceptance criteria. For modified-release drug products, *in vitro*/*in vivo* correlation (IVIVC) can be used to define *in vitro* acceptance criteria when *in vivo* bioavailability data in man are available for formulations exhibiting dissimilar *in vitro* release rates. Where such data are not available (which is often the norm) and drug release cannot be shown to be independent of *in vitro* test conditions (which again is often the norm), then *in vitro* acceptance criteria should be established on the basis of available batch data. Typically, the acceptable variability in mean *in vitro* release rates at any given time point should not surpass a total difference of ±10%, that is, a total variability of 20%. Therefore, a requirement of 20 ± 10% equates to an acceptable range from 10 to 30%), unless a wider range can be supported by appropriate *in vivo* studies (see decision tree 7(3)). The test is fully described in the pharmacopoeias (e.g., USP <711>, Ph. Eur. 2.9.3), and the release criteria are also defined (e.g., USP <724>, Drug Release)

(Continued)

Table 16.3 (Continued)

Drug product additional tests	Commentary
Hardness/ friability	Typically, hardness (breaking force) and/or friability testing are performed as in-process controls (see Table 16.1: In-Process Tests). Consequently, these attributes are not typically included on the drug product specification. However, if they are deemed to be CQAs, for example, chewable tablets, then appropriate acceptance criteria need to be included on the specification. The tests are fully described in all of the pharmacopoeias (e.g., hardness (USP <1217>, Ph. Eur. 2.9.9) and friability (USP <1215>, Ph. Eur. 2.9.7)
Uniformity of dosage units (solid oral dosage forms)	This can include either weight variability (WV) of the dosage form or content uniformity (CU) of the drug within the dosage form. These tests are harmonized (see ICH Q4) in the various national pharmacopoeias, for example, USP <905>, Ph. Eur. 2.9.40. However, there is some disagreement with respect to sample sizes. In these cases, Ph. Eur. has proposed an additional test for uniformity of dosage units for large sample sizes (2.9.47). In general, the specification should include one or the other, but not both test variants. Typically, the former (WV) is utilized for solid oral drug products with high API loading, and the latter (CU) is used where drug products have low drug loading. For the former (WV), applicants should demonstrate during drug development that drug product homogeneity is adequate (including homogeneity of halved-tablets, where the product has a functional break line). If appropriate, these tests may be performed either as an in-process test (with justification) or as a specification test
Microbial limits	If applicable, a microbial limit test should be included on the specification for non-sterile oral drug product (see Table 16.2: Microbial Limits for APIs for fuller details) Microbial limit testing is viewed as an aspect of GMP, as well as of testing and quality assurance. Microbiological risk assessments should be performed (USP <1115>, Ph. Eur. 5.1.4.,) to assess whether microbial limit tests need to be performed. Water also plays a key part in microbial growth, and it is recommended to assess the water activity of the formulation (USP <1112>, Ph. Eur. 2.9.39). Guidelines for microbial limits are provided by the pharmacopoeias (USP <1111>, Ph. Eur. 5.1.). Skip testing may be an acceptable approach where justifiable. (See decision tree 6 for microbial testing of excipients.) More recently, regulatory agencies [25] have embraced "fast-microbiology methods," allowing for rapid turnaround of testing and facilitating faster decision making [13]. The pharmacopoeias also have general chapters covering the area of fast microbiology testing (USP<1223>, Ph. Eur. 5.1.6). Decision tree 8 provides additional guidance on the use of microbial limits testing
Water content	If applicable, a water content test should be included on the specification for the oral solid drug product (see Table 16.2: Water Content for APIs for fuller details)

16.4.2.2 Drug Product (Solid Oral Dosage Forms) Specific Tests

The following specific tests are germane to solid oral dosage forms, for example, tablets (coated and uncoated) and hard gelatin capsules. Many of these tests will also be applicable to soft capsules and granules.

In addition, the following tests are also applicable to liquid and semisolid dosage forms, including sterile products (see Table 16.4).

Table 16.4 Additional specific tests for drug products (liquid/semisolid dosage forms).

Drug product additional tests	Commentary
pH	Acceptance criteria for pH (typically a range, rather than a specified value) should be provided where applicable and justified. The test is fully described in all of the pharmacopoeias (e.g., USP <791>, Ph. Eur. 2.2.3)
Water content	If applicable, a water content test should be included on the specification for the oral products requiring reconstitution and nonaqueous parenteral, for example, emulsions, colloids and liposomal preparations. A test and acceptance criterion for water content may be required. Test requirements are the same as for oral solid dosage forms (see Table 16.2: Water Content for APIs for fuller details)
Dissolution (oral suspension and for re-constitutable dry powder suspension products)	It may be appropriate for poorly soluble APIs to include dissolution testing for oral suspension formulations and for re-constitutable dry powder suspension products. Dissolution testing should be performed at release, on stability, and in support of planned changes. Test requirements are the same as for immediate release oral solid dosage forms. Acceptance criteria are based on the dissolution profiles of the batches that showed acceptable performance *in vivo* together with development and registration batches
Uniformity of dosage units (oral liquid and suspension dosage forms)	This test is applicable to either single-dose or multiple-dose containers of oral liquids (including sterile liquids) and oral suspensions. It should be noted that the dosage unit is considered to be the representative dose taken by the patient. If the dosage unit is controlled, it may either be measured directly or determined based on the total measured weight or volume of drug divided by the total number of doses anticipated. Note that the product label (e.g., *Undefined Oral Solution, 5 mg/ml*) defines whether the assessment is performed on an mg/g (weight) or mg/ml (volume) basis. If standard dispensing equipment, such as medicine droppers or dropping tips for bottles, is a fundamental part of the container closure system (preferred situation), then this dispensing equipment should be used to determine the dose. Otherwise, a standard volume measure should be used. The dispensing equipment to be used is normally determined during packaging development. Equally, for some sterile liquids, for example, prefilled syringes, the dispensing equipment is part of container closure system. For powders for reconstitution, weight variability (WV) testing is generally considered acceptable

(Continued)

Table 16.4 (Continued)

Drug product additional tests	Commentary
	Uniformity of dosage units is often performed in support of the in-use shelf life of the product (see ICH Q1). Thus, if a product is designed to deliver a 28 day course of treatment, then uniformity of dosage units (CU) should be performed over a 28 day period, that is, dosage unit 1 on day 1, dosage unit 2 on day 2, etc. This demonstrates that under normal dispensing conditions, the patient can deliver a homogenous, uniform product by following the prescribed dosing instructions
Microbial limits	If applicable, a microbial limit test should be included on the specification for the drug product (see Table 16.2: Microbial Limits for APIs for fuller details)
	Microbial limit testing is viewed as an aspect of GMP, as well as of testing and quality assurance. Microbiological risk assessments should be performed (USP <1115>, Ph. Eur. 5.1.4.) to assess whether microbial limit tests need to be performed. Water also plays a key part in microbial growth, and it is recommended to assess the water activity of the formulation (USP <1112>, Ph. Eur. 2.9.39). Guidelines for microbial limits are provided by the pharmacopoeias (USP <1111>, Ph. Eur. 5.1.). Skip testing may be an acceptable approach where justifiable. (See decision tree 6 for microbial testing of excipients.) Fast-microbiology methods may be applicable. The pharmacopoeias also have general chapters covering this area (USP<1223>, Ph. Eur. 5.1.6). Decision tree 8 provides additional guidance on the use of microbial limits testing
Antimicrobial effectiveness testing (AET) and preservative content	For oral liquids or suspensions that require the presence of an antimicrobial preservative(s) system, suitable acceptance criteria for the preservative(s) content should be justified. These acceptance criteria need to be evaluated based on the levels of antimicrobial preservative(s) required to maintain microbiological quality of the product throughout its shelf life (including its in-use shelf life). From a safety perspective, the lowest specified concentration(s) of antimicrobial preservative(s) required to be effective in controlling microorganisms needs to be determined. However, the lowest specified concentration(s) in the product may need to be slightly increased to address chemical and physical instability of the preservative(s) system throughout its shelf life
	AET is normally performed during pivotal stability studies at release, at the end of shelf life, at the end of the product's in-use shelf life, or when significant changes have been made to the drug product process, for example, during scale-up. Typically, assay of preservative(s) content is included on the specification, but AET is typically excluded. Under some circumstances, in-process testing of preservatives may suffice in lieu of release testing. However, when preservative content is performed as an in-process test, the acceptance criteria should remain an integral part of the release specification. Typically, release and end of shelf-life limits are broader than at release to reflect their greater intrinsic instability. The test is fully described in all of the pharmacopoeias (e.g., USP <51>, P h. Eur. 5.1.3)

Table 16.4 (Continued)

Drug product additional tests	Commentary
Antioxidant content	Release testing for antioxidant content(s) is typically required. Where justifiable, shelf-life testing may be unnecessary and where appropriate, in-process testing may be adequate instead of release testing. However, many antioxidants, for example, ascorbic acid, are sacrificial in nature, that is, they will oxidize in preference to API within drug product and hence need to be monitored and controlled. As per preservative (s) content, when antioxidant content testing is carried out as an in-process test, the acceptance criteria should remain part of the specification. If release testing is performed, this decision should be reevaluated whenever significant changes are made to the manufacturing procedure or there are changes to the container/closure system. Typically, release and end of shelf-life limits are broader than at release to reflect their greater intrinsic instability
Leachables[a]	This test is typically only determined for oral liquid and oral suspension products (where liquid is in continuous contact with the container/closure system throughout the products shelf life). For sterile liquids and suspensions the test is viewed as being critically important. For powders for reconstitution, extractable testing is not typically required. Indeed, where development and stability data have shown evidence that extractables from the packaging materials are typically lower than levels that are demonstrated to be acceptable and safe, elimination of this test can be typically endorsed. However, this should be reevaluated if the container/closure system or the formulation changes, i.e. major changes to the drug product. Where there is a demonstrable need (see Table 16.5), extractable testing of the container/closure system components (e.g., plastic bottle, cap liner, rubber stopper, etc.) are performed for oral solutions packaged in glass containers with non-glass closures, or in non-glass containers.
Alcohol content	Although new oral liquid/suspension products (particularly pediatric formulations) typically avoid the use of alcohol as a cosolvent, there may still be instances when alcohol will be required due to the poor intrinsic solubility of the API. In those cases, the alcohol content should be controlled on the specification. It is typically assayed (GC) or, less commonly, calculated. Typically, release and end of shelf-life limits are broader than for the API to reflect their greater variability
Particle size distribution	Developmental data should be evaluated when assessing the requirements for this test. Although particle size distribution is often used in lieu of dissolution for solid oral dosage forms, in the case of oral suspension products (or re-constitutable suspension products), the complexity of the formulation and the presence of insoluble excipients (e.g., suspending agents) make this approach analytically challenging, if not impossible. Note that particle size evaluation using analytical sieving is not appropriate for oral suspension products, although it may be applicable for re-constitutable suspension products

(Continued)

Table 16.4 (Continued)

Drug product additional tests	Commentary
	The acceptance criteria should include the percent of total particles in given size ranges. The mean, upper, and/or lower particle size limits should be appropriately defined. The potential for particle growth, that is, Ostwald ripening, should be assessed during product development using freeze/thaw cycling (see ICH Q1)
	Particle size distribution testing should be performed at release. In addition, it may be performed as an in-process test when justified by supporting data. However, if the product has been demonstrated to have consistently rapid release properties, then exclusion of this test from the specification may be proposed
	Unfortunately, there is no commentary in the ICH Q6A guidance on the critical impact of particle size for inhalation delivery, that is, lung delivery or intranasal delivery. However, the USP (and other pharmacopoeias) does have a general chapter covering this area, that is, USP <601>. There are also guidance documents from FDA [26] and EMA [27]. For more general background, interested readers should consult the relevant literature (e.g., Refs. [28–31])
Redispersibility	Oral suspensions are thermodynamically unstable and will typically sediment (or settle) on storage; therefore, acceptance criteria for redispersibility are typically required. The resuspension procedure should be defined to allow the patient to redisperse the product. In particular, the time necessary to achieve appropriate resuspension by the defined procedure should be clearly articulated (and fully described on product insert). Data generated during product development may be adequate to support intermittent testing (see Table 16.1: Periodic or skip testing), or removal of this test from the specification can be proposed
Rheological properties	For viscous oral liquids and particularly for suspension products, it may be appropriate to include a test for rheological properties, that is, viscosity and/or specific gravity on the product specification. Both the test and acceptance criteria should be defined. The test is fully described in all of the pharmacopoeias (e.g., USP <911>, Ph. Eur. 2.2.8). Viscosity can be determined using either a rotational rheometer test (e.g., USP <912>, Ph. Eur. 2.2.9) or a rolling ball viscometer test (e.g., USP <913>, Ph. Eur. 2.2.10). Skip lot testing or removal of this test from the specification can be proposed (see Table 16.1: Periodic or skip testing)
	For thixotropic suspensions this test has limited applicability as the intrinsic viscosity of the product is destroyed during the sampling/testing process (i.e., product is shear thinned to allow pouring). However, it may still be required by regulatory agencies as part of the regulatory submission. Applicants are recommended to discuss with regulatory agencies as part of the pre-submission process

Table 16.4 (Continued)

Drug product additional tests	Commentary
Reconstitution time	Appropriate acceptance criteria for reconstitution time should be provided for dry powder products or dispersible tablets (including effervescent tablets) requiring reconstitution. The reconstitution time needs to be patiently aligned (i.e., typically <2 min). The choice of diluent should be assessed and defined on product label. Factors affecting reconstitution times include porosity, solid-state form, degree of crystallinity, and wettability [32]. Skip lot testing or removal of this test from the specification can be proposed (see Table 16.1: Periodic or skip testing)
Sterility	Sterility testing (e.g., USP <71>, Ph. Eur. 5.1.9) is mostly harmonized across all pharmacopoeias. Sterility is also reliant on procedural measures that effectively prevent microbial contamination of sterile products, such as clean room technology and other GMPs. Where sterility data are generated during development and appropriate validation that justify parametric release, this strategy may be proposed for terminally sterilized drug products
Endotoxins/ pyrogens	Endotoxin testing, using a procedure such as the limulus amoebocyte lysate (LAL) test, is required for the specifications of sterile products (and APIs intended for sterile products). The test is fully described in all of the pharmacopoeias (e.g., USP <85>, Ph. Eur. 2.6.14)
	Pyrogenicity testing that is currently used for container/closures and medical devices/implants (e.g., USP <1031>, Ph. Eur. 2.6.8) may be proposed as a substitute to endotoxin testing
Particulate matter	Sterile liquid products, with the exception of sterile suspensions, should be tested for particulate matter. The test is fully described in all of the pharmacopoeias (e.g., USP <788>, Ph. Eur. 2.9.20)
	The specification will typically include acceptance criteria for visible particulates and/or clarity of solution, in addition to subvisible particulates, as considered appropriate. In those cases, where for technical reasons, the product cannot be tested by light obscuration methods (e.g., emulsions, colloids, and liposomal preparations), these products should be analyzed by microscopy methods (e.g., USP <788>, Ph. Eur. 2.9.19)
Functionality testing of parenteral delivery systems	Sterile products packed in prefilled syringes, auto-injector cartridges, or equivalent require test procedures and acceptance criteria related to the functionality of their delivery systems. These tests may include control of syringeability [33], applied pressure, seal integrity (as a measure of leakage), and/or assessment of "tip cap removal force, piston release force, piston travel force, and power injector function force." These tests may be performed as in-process tests. Skip lot testing or removal of some or all of these parameters from the specification can be proposed (see Table 16.1: Periodic or skip testing)

(Continued)

Table 16.4 (Continued)

Drug product additional tests	Commentary
Osmolarity	If the tonicity of a sterile product is declared in its labeling, then appropriate control of its osmolarity should be carried out. In-process control testing, skip lot testing (see Table 16.1: Periodic or skip testing), or using direct calculation of this attribute can be proposed

[a] referred to in the guideline as extractables, but the correct terminology is leachables.

Table 16.5 Overview of requirements for extractable/leachable testing.

Degree of concern associated with the route of administration	Packaging concerns for different classes of drugs		
	High	Medium	Low
Highest	Inhalation aerosols and solutions	Inhalation powders	
	Injections	Sterile powders	
	Injectable suspensions	Powders for injection	
High	Nasal aerosols and sprays		
	Ophthalmic solutions and suspensions		
	Transdermal ointments		
	Patches		
Low	Topical solutions and suspensions	Topical powders	Oral tablets
	Topical and lingual aerosols	Oral powders	Oral (hard and soft gelatin) capsules
	Oral solutions and suspensions		

Source: Adapted from FDA Guidance [34].

16.5 ICH Q6A Decision Trees

The ICH Q6A guidance contains eight decision trees aimed at helping applicants navigate the various courses of action necessary in establishing acceptance criteria. These are summarized in Figure 16.3.

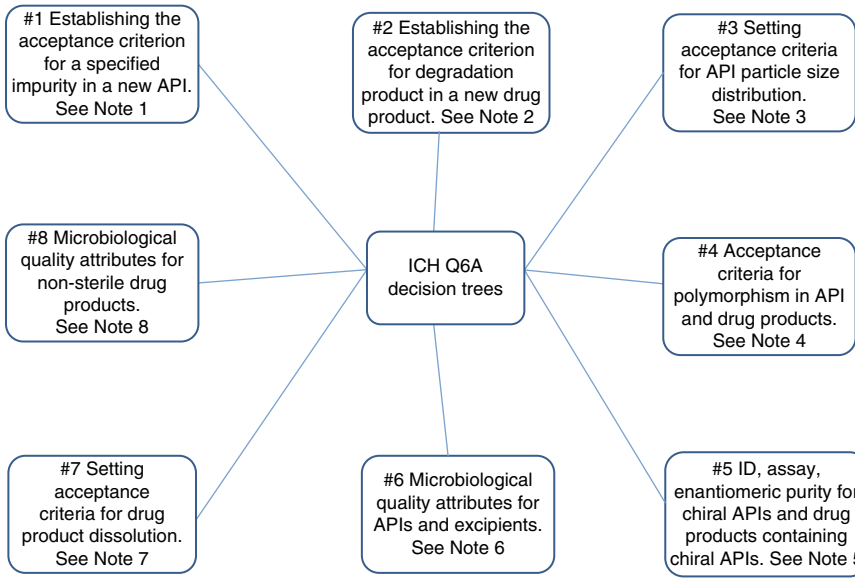

#1 Establishing the acceptance criterion for a specified impurity in a new API. See Note 1
#2 Establishing the acceptance criterion for degradation product in a new drug product. See Note 2
#3 Setting acceptance criteria for API particle size distribution. See Note 3
#8 Microbiological quality attributes for non-sterile drug products. See Note 8
ICH Q6A decision trees
#4 Acceptance criteria for polymorphism in API and drug products. See Note 4
#7 Setting acceptance criteria for drug product dissolution. See Note 7
#6 Microbiological quality attributes for APIs and excipients. See Note 6
#5 ID, assay, enantiomeric purity for chiral APIs and drug products containing chiral APIs. See Note 5

Figure 16.3 ICH Q6A decision tree overview
Note 1: See ICH Q3A [18]. Relevant batches are those synthesized during development, pilot, scale-up batches (and if available, commercial).
Note 2: See ICH Q3B [19]. Relevant batches are those manufactured during development, pilot, scale-up batches (and if available, commercial).
Note 3: May not be a CQA.
Note 4: May not be a CQA.
Note 5: Typically not required for drug substance.
Note 6: May not be a CQA.
Note 7: This discusses the comparative roles of disintegration and dissolution. This also discusses the acceptance criteria for single and multipoint dissolution specifications. Makes no mention of being a CQA.
Note 8: This is typically not required. Microbiological risk assessment (USP <1115> and Ph. Eur. 5.1.4) can be used to argue for the removal of the test or inclusion as a skip lot test.

16.6 Process Capability and Specification Setting

ICH Q6A does not include any discussions on process capability. However, it has become clear with the advent of QbD that a comprehensive understanding of the impact of variability of the process and supporting methodologies is required before robust specifications can be set.

Process capability (Cp) measures the output of an "in-control" process with the process specification limits measured using capability indices. This is done by assessing the ratio of the process specification width to the spread of process values using standard deviation units. As the process improves, the spread or variability tightens and it becomes more closely centered between the

Table 16.6 Process capability and out-of-specification (OOS) outcomes.

SD range	Process capability (Cp)	Probability that data are within specification limits	Out-of-specification (OOS) occurrence
6.00	1.00	99.73	1 in 370
7.55	1.25	99.98	1 in 5,655
9.00	1.50	99.993	1 in 147,150
12.00	2.00	99.9999998	1 in 5.1×10^8

specification limits. A process where the majority of all measurements reside within the specification range is a 6-sigma capable process. This is summarized in Table 16.6.

The proceeding discussion (see Table 16.6) has assumed that the data is centered within the specification range, that is, 100% for API potency, ± 2% for specified range, that is, 98.0–102.0%. However, it can be readily seen that this is not typically the case, and the presence of impurities means that the process is actually centered at 100–x%, where x is the total value of all impurities typically found in the process. This is reflected by the off-target process capability index (Cpk). A process with a Cp of 2 could have a Cpk of 1.5, which would equate to a failure rate of 4 defects in every million assessments. Thus logically process capability or variability is one of the key parameters in setting robust specifications.

However, regulators have indicated that it "was not considered appropriate to add method variability as determined in analytical method validation to the variation seen in batch results as this variability is already included in the batch results and therefore will be counted twice." [35] This view is predicated on the perspective that measurement uncertainty (MU) will always be smaller than batch variation. This is true if the sample size of the batches in question is sufficiently large to reflect the total population, which is typically a minimum of 30 batches [36]. However, in practice many new drug products will file with only three batches at scale, supplemented by a number of development batches at pilot scale. This leads to the key questions, "how will it be possible to ensure the variability of the production process is covered by the specification with a limited number of manufacturing scale lots that are available at the time of submission? Are these criteria reflected in the clinical experience with the product, or are the limits based on process performance alone [35]?"

Thus if the specification is artificially constrained and/or takes no account of process/method variability, it can turn a capable process into a non-capable process leading to OOS results. Unfortunately, this is an increasingly common occurrence during regulatory review, where reviewers think that by further constraining the proposed specification, they will make the process/

specification more discriminating and ensure patient safety—when in reality all that occurs are more batch failures of product that was of an acceptable quality. Let us examine this further using API assay specifications.

16.6.1 API Assay Specifications

An analytical methods' intermediate precision is the most appropriate method validation parameter for assessing Cp and therefore should be evaluated when proposing any specification limits, or when assessing the capability of the method when the specifications are constrained, as is the case for API assay, that is, 98.0–102.0%. It is widely accepted that method variability is frequently greater than manufacturing process variability, particularly for API processes. Tsang et al. [37] demonstrated that for any proposed API assay specification operating at 3σ, that is, process mean $\pm 3\sigma$, a specification of ±2% (4% range) is equivalent to a total variability of 0.67%; thus the method variability needs to be at least half this value, that is, 0.34% (or less). The allowable method variability is further constrained as the true mean of the specification is <100%, that is, 100 – (% impurities + % residual solvents + % water). Thus the methods need to show increased process capability (i.e., 6σ), and hence the total method deviation is required to be ≤ one-twelfth of the total allowable spread or tolerance [38], that is, 0.17% (or less). Even doubling the HPLC assay replicates still requires an analytical standard deviation of 0.64% (or less). Using an analytical range of 2% (100.0–98.0%, i.e., theoretical mean—lower specification limit) gives an allowable precision of 0.73 and 0.85% RSD, respectively. In the Ph. Eur., a maximum permitted HPLC system precision is defined for potency assays of API, which is reliant on both the upper API specification limit and the number of replicate injections in the method. Similarly, the FDA and Canadian guidance recommend HPLC system precisions of not >1% RSD (Figure 16.4).

From the perspective of generic allowable API specifications (98.0–102.0%), titrimetric methods do have process capability of $>6\sigma$, whereas typically most HPLC assay methods have process capability of only about 3σ.

Based on this significant HPLC method variability, and assuming an allowable API specification range of ±2.0%, several commentators [39–41] have expressed significant misgivings about the utility of the standard HPLC assay method to monitor drug substance quality. That is to accurately trend changes in API purity, to trend real changes in API quality on stability storage, to release API batches whose true potency is within the range 98.0–102.0% or reject batches whose true potency is out with that range, that is, <98.0%, or to meaningfully investigate OOS results that could be attributable to method variability not specification failures. Skrdla et al. [42] supported this view stating that "assay results are simply not stability-indicating (….) due to the large assay variability associated with them." Indeed, the influence of HPLC method variability on OOS results is also significantly impacted by FDA's 2006 [43] guidance,

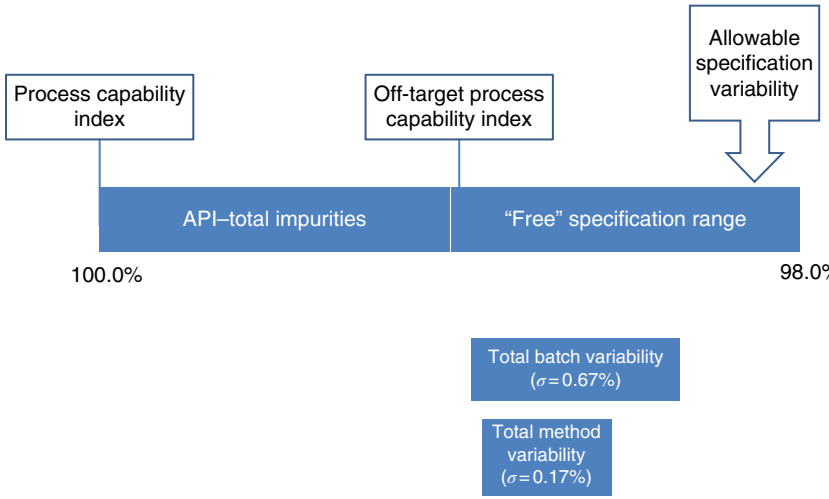


Figure 16.4 Schematic showing impact of impurities, process capability, and method capability on the allowable API specification variability.

which requires that "all individual sample replicates, as well as the average, fall within the acceptance criteria." Hofer et al. [40] used modeling to assess the probability of finding a false OOS and found that this was very dependent on the method variability and the true mean of the API batch. They also indicated that there was only a 1% chance of OOS results when the standard deviation was 0.6%, with a true mean of 99.4%, but this increased ninefold when the standard deviation increased to 1%, with the same true mean. The probability of seeing false OOS results also rises based on the total number of tests performed on the same batch, for example, during routine stability testing. Thus, if the true batch mean is 99.6% and the intrinsic method variability is modeled as being 0.6, 0.8 or 1.0% (RSD), respectively, then the probabilities of observing "false OOS" is relatively low, that is, 0.4, 2.4, or 6.3%, respectively. However, if this batch is placed on stability with 5 time points (0, 3, 6, 12, and 24 months) and independent duplicate assays are performed at each time point, that is, 10 assays in total, then the chances of obtaining a "false OOS" increase significantly. Therefore, using the same true mean and the same method variabilities (0.6, 0.8, or 1.0% RSD), the probabilities increase to 4, 22, and 49%, respectively. In summary, for a stable API placed on stability, it is still very likely that "false OOS" results "will be observed within a surprisingly small number of tests." The authors reflected that this was a significant waste of resources and may result in nonproductive countermeasures, as there is nothing intrinsically wrong with the batch in question, rather it is a statistical artifact of the method [40]. This of course can be addressed by registering wider specifications that

are based on process capability rather than on the much narrower specifications based on regulatory expectations (typically, 98.0–102.0%). However, it is unlikely whether these wider, more meaningful specification ranges would ever be accepted by regulatory reviewers.

Hofer et al. [40] reflected that the "HPLC assay is more a test of a laboratory's ability to achieve high precision than of drug substance quality" and compared the data from eight API batches using the standard external standard HPLC assays as opposed to the mass balance HPLC assay approach. Although, the mean assay data were similar (99.85 vs. 99.75%), the precision of the former data (pooled σ 0.55, range 0.31–0.80) was in the region of six- to eightfold higher than the corresponding mass balance HPLC assay approach (pooled σ 0.09, range 0.04–0.20). Indeed, Skrdla et al. [42] proposed the complete removal of the existing external standard HPLC percent assays from regular use within release or stability studies, replacing with the more precise mass balance HPLC assay approach. The authors claimed that the mass balance HPLC assay approach is better aligned to the current ICH reporting practices (<0.05%) for impurities and degradation products and that its introduction could lead to better trending and significantly less OOS reporting. However, in contrast, EMA have indicated that when analyzing both API and drug substance, an external standard method should be used (calculating % w/w) to evaluate and exclude any mass imbalance considerations. In contrast, area normalization may be acceptable for APIs that are comprised of a "family of compounds," for example, related antibiotics [43].

Without some regulatory relaxation of the current API specification limits of 98.0–102.0%, there seems little doubt that the use of the existing standard HPLC assay to monitor API quality must be approached with severe reservations. Tsang et al. [37] performed a retrospective analysis of the assay data for four different APIs from QC production laboratories, as well as R&D laboratories, and showed that the default 4% specification range, that is, 98.0–102.0%, did not allow for any meaningful variability within the registered process. Indeed, the authors reflected that the assay data would support the use of a 5% specification range, that is, 97.5–102.5%. They indicated that the quality of the API can be more accurately evaluated when HPLC potency data are evaluated holistically, with impurity data and other supporting data. Interestingly, this is the original concept of a pharmacopoeial specification (at least in Europe). The Ph. Eur [44]. indicates for specificity of assays that "For the elaboration of monographs on chemical active substances, the approach generally preferred by the Commission is to provide control of impurities (process-related impurities and degradation products) via a well-designed Tests section, with stability-indicating methods, rather than by the inclusion of an assay that is specific for the active moiety. It is therefore the full set of requirements of a monograph that is designed to ensure that the product is of suitable quality throughout its period of use."

16.7 Probability-Based Specification Limits

In a standard scenario, specifications have clearly defined acceptance and rejection zones (see Figure 16.5).

The USP and other pharmacopoeias are assessing and implementing decision rules based on a probabilistic assessment that acknowledges MU and the role it plays in accepting or rejecting a result based on its compliance with a predetermined specification [46]. MU is defined as "a parameter associated with the result of measurement that characterizes the dispersion of the values that could be reasonably attributed to the measurand." These decision rules support the decision making by assessing (i) the measurement result, (ii) the specification limit (both current practices), (iii) the MU, and (iv) the acceptable level of probability of making a wrong decision (both new processes). Further, an operating characteristic is not deemed significant if its standard deviation is one-third of the largest standard deviation. The specification decision rules will introduce the concept of a guard band or transition zone between the acceptance and rejection zones within a specification (see Figures 16.6).

Within the transition zone a product will be considered noncompliant if the probability of either being above or below the designated upper or lower specification limit exceeds 2.5%, and a target MU equivalent to a standard deviation of 1.02 would address these requirements. However, a bias caused by either the presence of impurities or a bias within the method itself will impact on this acceptable value. In the case of a bias of 1.0%, the standard deviation needs to be reduced to 0.6% in order to address these criteria. Further, a recent IUPAC/CITAC guide [47] provides advice on how to investigate OOS data based on MU considerations.

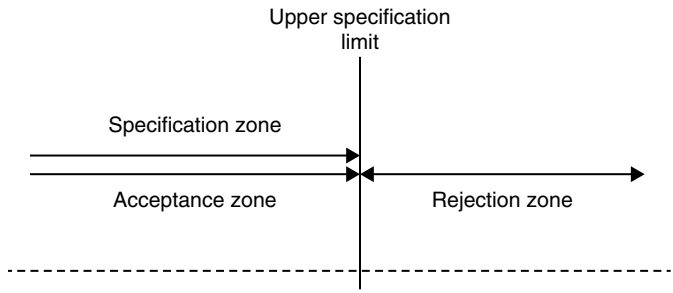


Figure 16.5 Specifications complying with simple acceptance criteria. Adapted from Ellison [45]. Reproduced with permission of Eurachem.

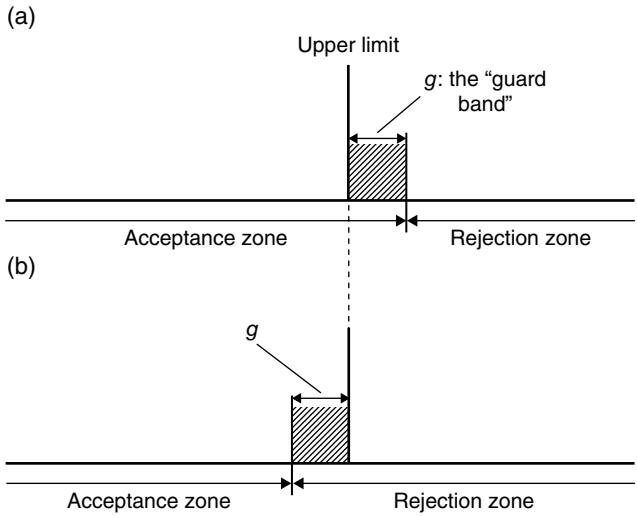


Figure 16.6 Specifications using acceptance criteria, (a) relaxed acceptance (test for nonconformity) and (b) stringent acceptance (test for conformity). Adapted from Ellison [45]. Reproduced with permission of Eurachem.

16.8 ICH Q6A and Quality by Design Initiatives (ICH Q8, Q9, Q10, and Q11)

Initially, the QbD [2, 48–50] initiatives were seen as having the potential to enhance many aspects of ICH Q6A [1], in particular to transform the existing reactive decision-making process to that based on a deeper understanding of the multivariant factors involved, to focus attention on designing processes to exceed current regulatory expectations and importantly, to understand critical variables, and to achieve a state of statistically based process control allied with the company's quality management system (QMS) to deliver continuous improvement [51]. More recently, it is not clear how the two initiatives are actively viewed and despite the ongoing dialogue, an updated ICH Q6A guidance will not be featured in the proposed new ICH initiatives. Indeed, a recent overview paper on QbD from FDA/academic authors makes no mention of ICH Q6A [52] nor indeed does a recent PQRI industry led group on the application of QbD to development, scale-up, and post-approval change control of IR and MR dosage forms [53]. Personal discussions with industry and regulatory thought leaders lead the author to believe that both QbD-based control strategies (design space, CQA, CPP, PAR (Proven Acceptable Range), etc.) and classical ICH Q6A specifications (in-process and release)

are both a subset of the Control Strategy; "a planned set of controls derived from current product and process understanding that ensures process performance and product quality [2]." Going forward, it is likely that clarification of the interrelationships between these guidances will emerge through the auspices of the implementation working group (QbD-IWG).

An increasingly common scenario that can occur in the new QbD paradigm is the setting of specifications based solely on the clinically derived CQAs, without taking into account process capability arguments (where process capability assessments clearly indicate that these proposed specification changes will make this a non-capable process, i.e., a 3-sigma process). A possible approach is to attempt to move toward the clinically derived specification in managed stages. Industry would therefore generate data from a meaningful number of batches, say, about 30 batches (allowing a realistic assessment of the population variability) and adjust the specification based on a revised process capability assessment. Then the final specification adjustments could be based on a assessment of process capability [54]. However, for some low volume products, these large numbers of manufactured batches are very aspirational.

16.9 Conclusion

The ICH Q6A guidelines have served industry and the regulators well over the last 15 years. However, the advent of the QbD guidelines has seen both parties struggling to understand the interrelationships and precedence of these guidelines.

Although it looked likely several years ago that ICH Q6A might be updated to better articulate the role of specifications in a QbD world, this will not occur in the near future. Instead, it looks increasingly likely that the QbD-IWG will need to clarify the position if the situation is to improve.

However, irrespective of the strength of the control strategy (underpinned by extensive understanding of critical process and quality attributes), if specifications are inappropriately tightened, then even the most highly capable processes (6-sigma) can be turned into failing 3-sigma processes. The relationship between the specification and the methods that underpin them is key, and to impose unrealistic specifications that necessitate the supporting methodologies operating beyond their design space, that is, tightening a dissolution specification from $Q = 80\%$ at 30 minutes to $Q = 80\%$ at 20 minutes, does not make the method more discriminating or bio-relevant, merely significantly less robust and likely to fail acceptable batches. That is, you increase OOS findings without materially improving patient safety, a "lose–lose" scenario. It is to be hoped that dialogue between all parties continues around this critical point.

References

1 ICH Q6A, 1999. Specifications: Test procedures and acceptance criteria for new drug substances and new drug products: Chemical substances. Current step 4 version, October 6, 1999.
2 ICH Q8(R2), 2009. Pharmaceutical development. Current step 4 version, August 2009.
3 ICH M7, 2014. Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. Current step 4 version, June 23, 2014.
4 ICH Q2(R1). Validation of analytical procedures: Text and methodology. Current step 4 version, November 2005.
5 ICH M3(R2), 2009. Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals. Current step 4 version, June 11, 2009.
6 Thomson NM, Seibert KD, Tummala S, Bordawekar S, Kiesman WF, Irdam EA, et al. 2014. Case studies in the applicability of drug substance design spaces developed on laboratory scale to commercial manufacturing. Organic Process Research and Development. 19(8); 925–934.
7 ICH Q6B, 1999. Specifications: Test procedures and acceptance criteria for biotechnological/biological products. Current step 4 version, March 10, 1999.
8 ICH Q4B. Evaluation and recommendation of pharmacopoeial texts for use in the ICH regions. Current step 4 version, November 1, 2007.
9 Pilot project on adaptive licensing, 2014. EMA/254350/2012, March 19, 2014.
10 FDA, 2014. Expedited programs for serious conditions, Drugs and Biologics, May 2014.
11 FDA, 2010. Guidance for industry submission of documentation in applications for parametric release of human and veterinary drug products terminally sterilized by moist heat processes. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), Center for Biologics Evaluation and Research (CBER), February 2010 CMC.
12 EC, 2015. Eudralex, the rules governing medicinal products in the European Union, Volume 4, EU guidelines for good manufacturing practice for medicinal products for human and veterinary use, Annex 17: Real time release testing, September 15, 2015 (Reference: Ares (2015) 3808922).
13 Duguid J, Balkovic E, du Moulin GC, 2011. Rapid microbiological methods: Where are we now? *American Pharmaceutical Review* 14(7). http://www.americanpharmaceuticalreview.com/Featured-Articles/37220-Rapid-Microbiological-Methods-Where-Are-They-Now/. Accessed on April 29, 2017.
14 ICH Q1B, 1996. Stability testing: Photostability testing of new drug substances and products. Current stage 4 version, November 6, 1996.

15 Hetrick EM, Vannoy J, Montogemery LL, Pack BW, 2013. Integrating tristimulus colorimetry into pharmaceutical development for color selection and physical appearance control: A quality-by-design approach. *Journal of Pharmaceutical Sciences* 102; 2608–2621.
16 ICH Q3C(5), 2011. Impurities: Guidelines for residual solvents. Current step 4 version, February 4, 2011.
17 ICH Q3D, 2014. Guideline for elemental impurities. Current step 4 version, December 15, 2014.
18 ICH Q3A(R2), 2006. Impurities in new drug substances. Current step 4 version, October 25, 2006.
19 ICH Q3B(R2), 2006. Impurities in new drug products. Current step 4 version, June 2, 2006.
20 FDA, 2015. Biopharmaceutics system (BCS) guidance. http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm128219.htm. Accessed on May 16, 2015.
21 Al-Gousous J, Langguth P, 2014. Oral solid dosage form disintegration testing—The forgotten test. *Journal of Pharmaceutical Sciences* 104; 2664–2675.
22 FDA, 2015. Dissolution testing and specification criteria for immediate-release solid oral dosage forms containing biopharmaceutics classification system class 1 and 3 drugs guidance for industry. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), August 2015, Biopharmaceutics.
23 SUPAC MR, 1997. Modified release solid oral dosage forms. Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Dissolution Testing and In Vivo Bioequivalence Documentation. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER).
24 EMA, 2014. Guideline on quality of oral modified release products, March 20, 2014, EMA/CHMP/QWP/428693/2013 Committee for Medicinal Products for Human Use (CHMP).
25 Störmer M, Vollmer T, Henrich B, Kleesiek K, Drier J, 2009. Broad-range real-time PCR assay for the rapid identification of cell-line contaminants and clinically important mollicute species. *International Journal of Medical Microbiology* 299; 291–300
26 FDA, 2002. Nasal spray and inhalation solution, suspension, and spray drug products—Chemistry, manufacturing, and controls documentation, July 2002. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm070575.pdf. Accessed on July 27, 2015.
27 EMA, 2006. Guideline on the pharmaceutical quality of inhalation and nasal products, June 21, 2006, EMEA/CHMP/QWP/49313/2005 Corr. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003568.pdf. Accessed on July 27, 2015.

28 Shekunov BY, Chattopadhyay P, Tong HHY, Chow AHL, 2007. Particle size analysis in pharmaceutics: Principles, methods and applications. *Pharmaceutical Research* 24; 203–227.

29 Patton JS, Byron PR. 2007. Inhaling medicines: Delivering drugs to the body through the lungs. *Nature Reviews Drug Discovery* 6; 67–74.

30 Labiris NR, Dolovich MB. 2003. Pulmonary drug delivery. Part I: Physiological factors affecting therapeutic effectiveness of aerosolized medications. *British Journal of Clinical Pharmacology* 56; 588–598.

31 Crowder TM, Rosati JA, Schroeter JD, Hickey, AJ, Martonen TB, 2002. Fundamental effects of particle morphology on lung delivery: Predictions of Stokes' Law and particular relevance to dry powder inhaler formulation and development. *Pharmaceutical Research* 19; 239–245.

32 Hiwali H, Amin A, Kumar L, Bansal AK, 2008. Variables affecting reconstitution time of dry powder injection. *Pharmaceutical Technology*, July 2, 2008. https://www.ncbi.nlm.nih.gov/pubmed/19921439. Accessed on April 29, 2017.

33 Kumar R, Palmieri MJ, 2010. Points to consider when establishing drug product specification for parenteral microspheres. *AAPS Journal* 12; 27–32.

34 FDA, 1999. Container closure systems for packaging human drugs and biologics, May 1999. https://www.fda.gov/downloads/drugs/guidances/ucm070551.pdf. Accessed on April 29, 2017.

35 EMA, 2011. Report on the expert workshop on setting specifications for biotech products, European Medicines Agency, London, September 9, 2011, EMA/CHMP/30584/2012.

36 Porter WR, 2012. Impact of stability on setting and meeting specifications in an uncertain world, MSBW 2012. http://www.mbswonline.com/upload/presentation_5-25-2012-10-43-30.ppt. Accessed on July 27, 2015.

37 Tsang PKS, Larew JSA, Larew LA, Miyakawa TW, Hofer JD 1998. Statistical approaches to determine analytical variability and specifications: Application of experimental design and variance component analysis. *Journal of Pharmaceutical and Biomedical Analysis* 15; 1125–1141.

38 Dejaegher B, Jimidar M, De Smet M, Cockaerts P, Smeyers-Verbekea J, Vander Heydena Y, 2006. Improving method capability of a drug substance HPLC assay. *Journal of Pharmaceutical and Biomedical Analysis* 42; 155–170.

39 Ermer J., 2001. Validation in pharmaceutical analysis. Part I: An integrated approach. *Journal of Pharmaceutical and Biomedical Analysis* 24; 755–767.

40 Hofer JD, Olsen BA, Rickard EC, 2007. Is HPLC assay for drug substance a useful quality control attribute? *Journal of Pharmaceutical and Biomedical Analysis* 44; 906–913.

41 Ermer J, 2005. *Method Validation in Pharmaceutical Analysis. A Guide to Best Practice*. In: Ermer J, Miller JHMcB, Eds. Wiley-VCH GmbH and Co., KGaA, Weinheim, p. 88.

42 Skrdla PJ, Wang T, Antonucci V, Dowling T, Ge Z, Ellison Det al. 2009. Use of a quality-by-design approach to justify the removal of the HPLC weight %

assay from routine API stability testing protocols. *Journal of Pharmaceutical and Biomedical Analysis* 50; 794–796.

43 EMA, 2014. Guidelines on setting specifications for related impurities in antibiotics, June 30, 2012, EMA/CHMP/CVMP/199250/2009corr.

44 Ph. Eur. 2.2.46. Chromatographic separation techniques. http://library.njucm.edu.cn/yaodian/ep/EP5.0/02_methods_of_analysis/2.2__physical_and_physicochemical_methods/2.2.46.%20Chromatographic%20separation%20techniques.pdf. Accessed April 29, 2017.

45 Ellison, SLR, Williams, A. (Eds). 2007. Eurachem/CITAC guide: Use of uncertainty information in compliance assessment. Available from http://www.eurachem.org. Accessed on July 24, 2017.

46 Weitzel J., 2014. Lifecycle management of analytical procedures: What is it all about? http://www.aaps.org/uploadedFiles/Content/Sections_and_Groups/Regional_Discussion_Groups/Southern_California_Pharmaceutical/Resources/Applying%20Lifecycle%20Approach%20to%20a%20Method%20rev%202.pdf. Accessed on September 2, 2014.

47 IUPAC/CITAC. 2012. Investigating out-of-specification test results of chemical composition based on metrological concepts (IUPAC technical report). *Pure Applied Chemistry* 84; 1939–1971.

48 ICH Q9, 2005. Quality risk management. Current step 4 version, November 9, 2005.

49 ICH Q10, 2008. Pharmaceutical quality system. Current step 4 version, June 4, 2008.

50 ICH Q11, 2012. Development and manufacture of drug substances (chemical entities and biotechnological/biological entities). *Current step 4 version*, May 1, 2012.

51 Hussain AS. What impact should ICH Q8 have on ICH Q6A decision trees? http://www.fda.gov/ohrms/dockets/ac/05/slides/2005-4187s1_10_hussain.ppt. Accessed on August 30, 2014.

52 Yu LX, Amidon G, Khan MA, Hoag SW, Polli J, Raju GK, et al. 2014. Understanding pharmaceutical quality by design. *AAPS Journal* 4; 771–783.

53 Van Buskirk GA, Asotra S, Balducci C, Basu P, DiDonato G, Dorantes A, et al. 2014. Best practices for the development, scale-up, and post-approval change control of IR and MR dosage forms in the current quality-by-design paradigm. *AAPS PharmSciTech*. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4037495/. Accessed on August 30, 2014.

54 Moran E, Perry M, 2011. Setting specifications, statistical considerations, EuropaBio Meeting. http://www.ema.europa.eu/docs/en_GB/document_library/Presentation/2011/10/WC500115818.pdf. Accessed on September 4, 2014.

17

ICH Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/ Biological Products

Scott R. Rudge and Raymond W. Nims

RMC Pharmaceutical Solutions, Inc., Longmont, CO, USA

17.1 Introduction

Typically, a specification lists a set of product attributes, a test for each attribute, a reference to an analytical procedure for the test, and appropriate acceptance criteria for the analytical procedure. The specification establishes the criteria by which a drug product (DP), drug substance (DS), raw material, intermediate, or other materials associated with pharmaceutical manufacturing are assessed for acceptability for their intended uses. The establishment of specifications is therefore a critical part of an overall control strategy, one that helps to ensure process consistency and quality of a product.

Specifications are required by regulatory agencies as part of a marketing application for a product. The International Conference on Harmonisation (ICH) Q6B document provides guidance for establishing specifications applicable to commercial biological products but specifies in the scope that it does not "apply to the regulation of preclinical and/or clinical research material" [1]. In practice, however, submission of an Investigational New Drug Application (IND) or an Investigational Medicinal Product Dossier (or equivalent European Union (EU) or Japanese document for which the ICH standards have been harmonized) also requires a list of analytical methods and associated acceptance limits to ensure that the identity, strength, quality, and purity of the drug meet expectations [2]. Analytical methods and associated acceptance criteria are also required by the European Medicines Agency (EMA) for biological investigational medicinal products to be used in clinical trials [3]. Specifications for clinical products are therefore needed as well, and typically the ICH Q6B guidance is followed for creating these.

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The current version of the Q6B guideline was adopted into the regulatory bodies of the EU, Japan, and the United States in 1999. Since then, various additional guidelines have been published, which supplement Q6B and specifically address the challenges of establishing specifications during drug development [4–7]. Many of these newer guidance documents refer back to Q6B, and it is therefore evident that the principles in the Q6B guideline can be applied to the development of clinical specifications. The principles to be considered in setting specifications per ICH Q6B are discussed in subsequent sections of this chapter.

The following points discussed in Q6B should be taken into account when establishing scientifically justifiable specifications:

- Specifications should be linked to a specific manufacturing process.
- Specifications should account for the stability of DS and DP.
- Specifications should be linked to preclinical and clinical studies.
- Specifications should be linked to the performance capabilities of the associated analytical methods.

The amount and quality of supporting data for establishing specifications may be limited during the early stages of product development. For phase 1 specifications, initial acceptance criteria are often based on a limited number of development batches used in nonclinical studies and involving analytical methods that have not yet been validated. Stability data may also be very limited at this phase of product development. Although data may be limited, careful consideration should be given to the quality attributes that may impact patient safety. All available information, including product type, planned dose and route of administration, and duration of dosing, should be taken into account when establishing specifications for such attributes.

In cases where the available data may be too limited to establish a relevant acceptance criterion for a given product characteristic, the attribute should still be included on the specification, perhaps with an acceptance criterion of "report results." On the other hand, preliminary specifications may be expected by the regulatory agencies for biologics that are well characterized [4]. In these instances, preliminary specifications may be established on the basis of a company's experience with a particular class of molecules and/or published information.

The acceptance criteria assigned to various attributes may be broader during the early stages of product development due to the limited availability of data. The specification may evolve during the product development life cycle as more data become available for a given attribute. Regulatory agencies acknowledge this and also understand that modifications to the manufacturing processes for a new drug entity are likely during development. Therefore, the initial specifications may not be fully reflective of process capability. Furthermore, the final product formulation often is not settled upon until clinical phase 3, at which time additional product attributes (e.g., pH, volume in container, osmolality, and tests for functional excipients) may be added to

the specification. It is for these reasons that finalized product specifications are not required by regulatory agencies until submission of the marketing application.

As drug development continues into clinical phase 2 and phase 3, more data become available, and it is expected that the specifications will be reviewed and updated as required. The need for specification revision may be driven by information obtained from process development manufacturing activities, analytical method development activities, and data generated from stability studies. All of these information sources must be considered when reviewing the specification to ensure it remains relevant. Any changes to the specification must be documented and a scientific justification provided. It should be kept in mind that removal of attributes and broadening of acceptance criteria for an attribute at the later stages of product development could result in added scrutiny from regulatory agencies. The expectation is that as product development progresses, specifications for the various attributes will "be refined" with acceptance criteria potentially tightened and the justifications for each criterion substantiated with batch data [6].

Going into phase 3 clinical studies, it is expected that the final manufacturing processes should be well defined, the final formulation implemented, and the validation of analytical methods nearly complete. These factors, together with clinical, manufacturing, and stability data, should provide adequate information to establish specifications that are close to those that will exist at the time of submission of the marketing application. Acceptance criteria should be tightened if appropriate, and the justification of the specifications enhanced. In-process specifications should also be implemented by this time. These specifications will be used to support process validation. Once the phase 3 specifications are established, any additional changes should be to simply enhance the justification to support the marketing application.

It is clear from the aforementioned discussion that specifications typically evolve over the course of the life cycle of a product. Specifications may even require further refinement post-marketing, as additional commercial manufacturing data become available. At the end of this chapter, case studies will be presented that discuss in further detail the establishment of specifications for a monoclonal antibody DP at an early stage of product development as well as a plasmid DP at a later stage of product development.

17.2 Principles to Be Considered in Establishing Specifications

Knowledge of the structure and function of a molecule is essential for setting meaningful specifications. Biological molecules have four levels of chemical structure, known conveniently as primary, secondary, tertiary, and quaternary (Figure 17.1). Primary structure refers to the amino acid sequence of the

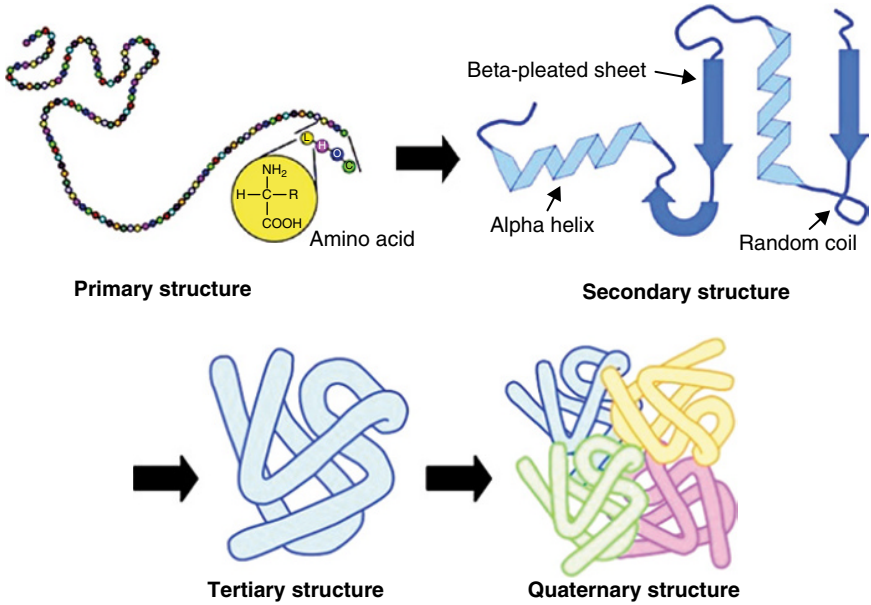


Figure 17.1 Four levels of protein structure.

molecule or the sequence of nucleotides in DNA or RNA drugs. The secondary structure is the structure conferred upon the molecule by intra-chain covalent bonds, primarily disulfide bonds between cysteines, and glycosylation. There are no analogous structural features in polynucleotides. The tertiary structure is the structure of the folded molecule. In proteins, this consists of alpha helices and beta-pleated sheets primarily. In polynucleotides, this consists of intra-chain base pairing and coiling.

Finally, quaternary structure is the description of subunit interactions, in which two or more molecules, either identical or different, combine to form the final active molecule. Some example methods for determining these four levels of structure are shown in Table 17.1.

The importance of these measurements to product quality is determined on a case-by-case basis. The attributes that are selected for inclusion within a specification should provide a high level of assurance that all the structural features of the molecule are consistent from lot to lot and are preserved during storage in stability studies.

For a polynucleotide product, such as a plasmid or an antisense polynucleotide, provided that the nucleotides are not chemically altered to improve stability (usually the case with synthetic polynucleotides), the list of methods typically employed is much smaller. Typically, sequencing and restriction enzyme analysis are sufficient to show primary structure, and some form of

Table 17.1 Analytical methods used for determining structure of therapeutic proteins.

Analytical method	Level of structure
N-terminal amino acid sequencing	Primary
Peptide mapping (reduced and nonreduced)	Primary and secondary
Mass spectrometry	Primary
Glyco-HPLC	Secondary
Peptide mapping with MS and MS/MS	Primary and secondary
Disulfide content	Secondary
FTIR spectroscopy	Tertiary
Second derivative UV spectroscopy	Tertiary
NMR	Tertiary and secondary
Differential scanning calorimetry	Tertiary and quaternary
Size-exclusion chromatography	Quaternary
SDS-polyacrylamide gel electrophoresis (reduced and nonreduced)	Quaternary

FTIR, Fourier-transform infrared; HPLC, high performance liquid chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance; UV, ultraviolet.

HPLC or electrophoresis that distinguishes between supercoiled forms, open circular forms, and linear forms is used to determine tertiary structure. Additional types of methods may be employed for DNA- and RNA-based therapeutics.

17.3 Content of Specifications

The Q6B guideline discusses product quality attributes associated with the DS and DP manufacturing steps. The quality attributes normally included in DS and DP specifications are almost identical, with two exceptions. First, a DS specification typically includes tests for process impurities (see also Chapter 18 for a deeper discussion of this topic), whereas such tests are usually not found in a DP specification. The second exception is that a DP specification normally includes tests specific to the final dosage formulation (such as pH, volume in container, sterility, osmolality, and tests for functional excipients), while such testing is not typically found in a DS specification.

As explained in the introduction, specifications are initially developed to support phase 1 first-in-human (FIH) studies. Tests for quality, identity, purity, potency, and safety are necessary to ensure proper control of the manufacture of the DS or DP [2]. Safety tests should include tests for process impurities (e.g., host cell DNA, host cell protein, and Protein A if applicable), tests for adventitious agents such as microbes and viruses, and a test for bacterial endotoxin.

The initial specification should start from the target product profile (TPP), as defined in ICH Q8 [8]. The TPP addresses the initial design requirements of the product, including the intended use in the clinic, the route of administration, the dosage form and strength, the container closure, the desired/required pharmacokinetic characteristics, and quality criteria such as sterility, intended storage condition, and purity requirements. This document forms the basis of the design of the product for the patient population for which it is intended and is the foundation of "quality by design" (QbD).

The initial specification is further refined based on predicted quality attributes and acceptance criteria for the given class of molecule, company experience, industry knowledge, and regulatory guidance. This typically takes into account an assessment of available toxicology, process, analytical characterization, analytical method performance, stability, and formulation information. Depending on the philosophy of the organization, either these attributes may be placed on the specification without limits, or an "internal specification" may be set, which is not immediately reported to regulatory agencies.

All attributes should be reassessed for suitability as additional product and process data become available, especially if process changes are implemented. In support of the marketing application, attributes should be either defined on the specification with acceptance criteria or removed from the specification based on supportive data that the attribute is deemed to have no safety and/or potency risk. As mentioned previously, removal of attributes from a specification could result in added scrutiny from regulatory agencies. An example of the refinement of a DS specification during evolution from early-phase to later-phase product development is depicted in a case study at the end of this chapter.

Critical quality attributes (CQAs) are physical, chemical, biological, or microbiological attributes or characteristics that must be within an appropriate limit, range, or distribution to ensure the desired product quality. The inability to define the attributes that are critical to product quality, or to establish acceptance criteria for CQA, can pose safety and efficacy risks [8]. In addition, "differences in CQAs that are within specifications but outside of clinical experience may have unknown but potentially significant consequences" [7]. A best practice is therefore to assess the criticality of product quality attributes to mitigate safety and efficacy risks per the principles described in ICH Q9 [9]. A risk assessment is an evaluation of the probability of occurrence of harm and

the severity or consequence of that harm. Product quality attributes may be assessed for impact to safety and efficacy. The severity of the impact, the likelihood of occurrence, and the detectability of the occurrence are then evaluated using, for instance, failure modes and effects analysis (FMEA), and then the results are ranked based on a predefined set of rules. The risk assessment helps to define the CQAs that must be monitored/controlled and establishes the rationale for the proposed initial specification limits.

Table 17.2 outlines the quality attributes and tests typically associated with a therapeutic protein product.

The product attributes are typically divided into a number of categories, including identity, quality (sometimes referred to as "characteristics"), assay or potency, purity/impurities, and microbial or adventitious agent safety. As pointed out in the Identity section of Q6B, "More than one test (physicochemical,

Table 17.2 Quality attributes and tests typically associated with a therapeutic protein product.

Manufacturing step	Quality attribute	Tests
DS/DP	Concentration	UV absorbance at 280 nm
DS/DP	Appearance/color/clarity	Visual
DS/DP	Purity/product-related impurities (charge variants, aggregates, clips, misfolding, truncations, etc.)	RP-HPLC, SEC-HPLC, CEX-HPLC, SDS-PAGE, CE-SDS, cIEF
DS/DP	Identity	RP-HPLC, IEX-HPLC, cIEF
DS/DP	Potency	Bioassay, ELISA
DS/DP	Freedom from adventitious agents (bacterial endotoxins, viruses, sterility, bioburden, mycoplasma)	USP <61>,<62>,<63>, <77>, <85>, Ph. Eur., JP, *in vitro* virus
DP	General characteristics	pH,[a] volume, osmolality,[a] volume in container, subvisible particles, functional excipients
DS	Process-related impurities: host cell DNA, host cell protein, protein A (if applicable)	qPCR, ELISA, SDS-PAGE

CE-SDS, capillary electrophoresis on sodium dodecyl sulfate; CEX-HPLC, cation-exchange high performance liquid chromatography; cIEF, capillary immunoelectrophoresis; DP, drug product; DS, drug substance; ELISA, enzyme-linked immunosorbent assay; JP, Japanese Pharmacopoeia; LAL, *Limulus* amebocyte lysate; Ph. Eur., European Pharmacopoeia; qPCR, quantitative polymerase chain reaction; RP-HPLC, reversed-phase high performance liquid chromatography; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SEC-HPLC, size-exclusion high performance liquid chromatography; USP, Unites States Pharmacopeia.

[a] pH and osmolality are also tests commonly found on DS specifications.

biological and/or immunochemical) may be necessary to establish identity." [1] This holds true for establishing purity and potency as well. The complexity of the molecule and the availability of multiple appropriate orthogonal analytical methods should be considered. For example, three tests may be necessary to define purity: a SEC-HPLC test may be used to detect aggregates, a CEX-HPLC test for detecting charge variants, and RP-HPLC or electrophoresis such as CE-SDS for the detection of misfolded variants (Figure 17.2). Similarly, potency for a monoclonal antibody product may be defined by the ability to bind an antigen and to stimulate cell apoptosis, requiring one ELISA assay and one

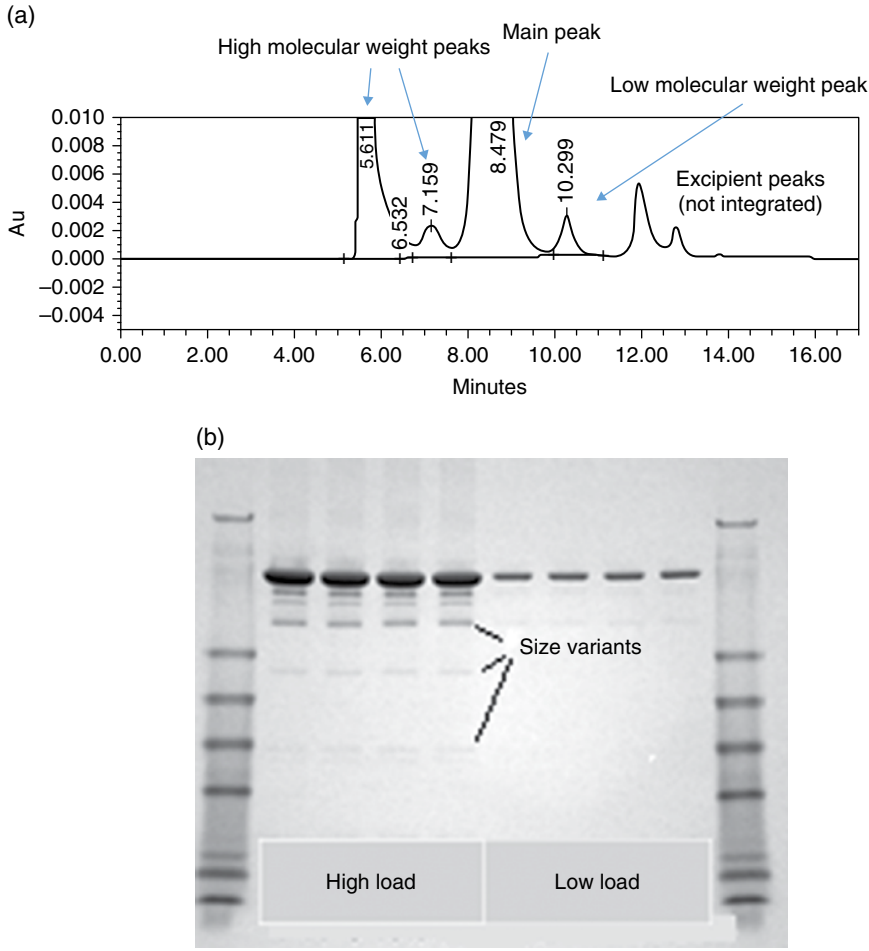


Figure 17.2 Demonstration of size variants using SEC-HPLC (a) and SDS-PAGE (b) and charge variants using CEX-HPLC (c) and cIEF (d).

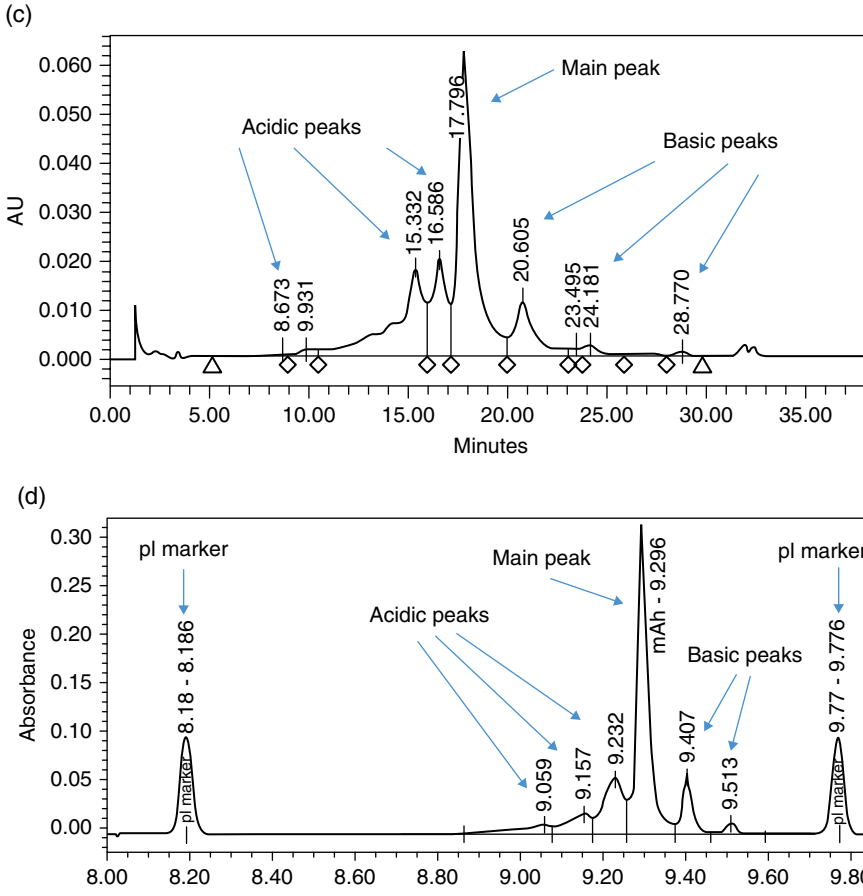


Figure 17.2 (Continued)

cell-based assay to establish potency in early phases. Multiple potency assays are also typically found with gene therapy products, where the guidance suggests that transfection efficiency, expression, and activity of the expressed molecule each must be demonstrated [5].

The Q6B guidance also states in section 4.2.3, Purities and Impurities, "If impurities are qualitatively and quantitatively (i.e., relative amounts and/or concentrations) the same [in the DP] as in the drug substance, testing is not necessary." [1] This guidance is applied in the case of host cell-related impurities such as host cell proteins and DNA content as sources of these impurities are absent in DP processes. For other types of impurities, often the composition and manufacturing process of the DS and DP are not the same in ways that influence their levels. Even if the DS and DP are similar, little data typically are

available regarding stability of the DP in the early phases of drug development. Therefore, the tests for purity and process-related impurities to be listed on the DS specification are often repeated on the DP specification, with the same limits.

Final specifications are based on detailed product and process characterization, manufacturing data, stability, and clinical experience. The acceptance criteria should be based on statistical analysis of lots tested by relevant and validated analytical methods.

17.4 Reference Standards

Reference standards must be developed for all new products from the time of early development. Typically, a reference standard consists of material segregated from an early lot, usually a lot used for IND-enabling toxicology testing. For biotechnology products, there is usually no additional purification conducted on the reference standard sample. However, the reference standard is usually stored in smaller quantities at lower temperatures (e.g., 1 ml cryovials at −80°C) than would normally be used for the DS or DP. It is not important whether reference standard is derived from DS or DP, as long as these are similarly formulated. If there is a preference, it is usually for the more dilute material in order to preserve precious early-stage materials. It is not uncommon, for instance, for a reference standard to be further diluted from a sample from DS lots, as analytical requirements are small and the reference standards are typically further diluted out prior to use.

As described previously, although the reference standard represents the same active pharmaceutical ingredient as that in DS or DP, it is different in material ways from either. To summarize, these are some of the possible differences:

- Storage temperature
- Storage container
- Concentration
- Volume in container

Because of these differences, reference standard stability often must be evaluated in a separate study. The stability test need not be according to ICH Q5 [10] standards, it need only be scientifically sound. For example, it is not unusual that testing is conducted only on an annual basis. It can be difficult to design good reference standard stability tests as well, as the only valid comparator may be reference standard itself. Usually, the most important tests for reference standard are those for content, structural integrity, and biological activity. If available, biological activity for a reference standard can be measured in comparison with a material supplied by a third party.

17.5 Method Validation

All analytical methods must be validated for inclusion in the marketing license application (Common Technical Document (CTD), Biologics License Application (BLA), or New Drug Application (NDA)). However, during the development of a product specification, the analytical methods are typically not fully validated. The term "qualification" is often used to define a stage of testing that provides data concerning the suitability of the method, without having fully validating the method. The term "phase appropriate validation" is also being used to mean the same thing as qualification. The extent of method qualification required in early stages of product development is left to the discretion of the developer, with the exception of safety tests such as bioburden, sterility, and endotoxin testing. The latter compendial methods are normally expected to be verified per USP <1226> [11] prior to testing a new biologic in humans.

Since there is discretion in the qualification protocol for non-safety methods in early-phase development, practice varies widely in the industry. For content and purity methods, accuracy and precision minimally are tested. When the purity method is a limit test, the limit of detection and limit of quantification are typically determined. Sometimes, particularly for product-related impurity methods, there is a test for sample stability. Usually, the effect of the sample matrix on the analytical method is tested. For identity and activity methods, specificity is an important parameter, especially if there is a possibility for a homodimer or heterodimer possessing different activity from the monomer (or vice versa, depending on the product). There is little, if any, qualification of simple compendial methods such as pH and osmolality expected during early-stage development.

During qualification, protocols are written, but acceptance criteria quite often are omitted or are very wide. As the performance of the assay is typically not well characterized at this point, this is an acceptable practice. However, if the performance as measured calls the veracity of any given method into question, further development may be needed, even if acceptance criteria were not violated.

17.6 Process Controls

Process controls may be used as a surrogate for release testing in some cases. Reducing the release testing of pharmaceutical products in favor of process control is a stated aim of the process analytical technology (PAT) [12] and QbD [13] initiatives. The general concept is to establish, through data, that a product that meets a certain standard by a certain point in the process will obligatorily meet the agreed release standard as a final product. A common

example is the water content achieved during a drying step monitored by infrared spectroscopy online. If the water content is at or below the acceptable level in the drier, and the material is not significantly hygroscopic, then achieving the release specification is assured and water content need not be tested again at lot release. Another example frequently given is the level of host cell protein in a protein product. In-process testing can establish a log clearance for host cell protein through each step in the process, and if in-process testing establishes the typical performance of the unit operations, it may be acceptable to exclude host cell protein testing from the release specification for the DS.

Testing for adventitious agents (bacteria, mycoplasma, viruses) in cell culture harvest samples is an example of an in-process control that can affect the release of the batch. Adventitious organism testing is typically performed on unprocessed harvest samples, which typically contain intact or lysed cells at the limit of their "*in vitro* age." These samples are tested for the presence of viruses, bacteria, and mycoplasma that otherwise should not be present in the cell culture. If the presence of one of these contaminants is confirmed, usually the batch must be rejected. Practice varies across the industry as to whether these tests formally appear on a final product specification. However, failure to meet a biosafety standard in process should result in the failure of the batch.

17.7 Raw Materials

Raw material specifications are expected to be defined for all process additives (e.g., buffers, media components, excipients), process aids (e.g., flocculants, precipitation agents, diatomaceous earth), and all product-contacting materials such as filters and container/closure components. Typically, compendial (i.e., USP or European Pharmacopoeia) specifications are sufficient. Complex raw materials (meaning diverse and/or poorly characterized mixtures) are generally not compendial. Setting specifications that have process significance for these types of raw materials may be difficult. For example, yeast extracts and soy hydrolysates often contain amino acids and vitamins that may impact the growth and productivity of the production cells, as well as the purity of the protein products being expressed. However, these raw materials are typically used in overabundance, and the production cells have pathways for interconverting the various substrates. The required limits for any single amino acid or vitamin are therefore unknown and, in a practical sense, unknowable. Because of this, testing for levels of specific amino acids and vitamins has generally been found to be unproductive. Nevertheless, justifications are generally expected for all non-compendial raw material specifications.

17.8 Case Study: Early-Stage Therapeutic Monoclonal Antibody

Establishment of specifications for antibodies produced from CHO cells is fairly straightforward. As antibody technology is constantly evolving and improving, the reader is advised to seek up-to-date references for benchmarking. Table 17.3 lists a set of specifications established for a hypothetical monoclonal antibody DP to be entered into a phase 1 clinical trial. The rationale for inclusion of the various attributes and limits is discussed in the succeeding text.

17.8.1 Identity

The ultimate test for the identity of an antibody is demonstration of its specific affinity for the antigen of interest. This appears to be the current standard for antibody identity tests. Some additional methods for demonstrating the affinity of an antibody for its antigen are ELISA, protein dot blotting, affinity beads, and Western blot. Of these methods, perhaps the protein dot blot and the affinity beads are the most convenient. Whichever method is used, there should be a test for binding of the antibody, another test for nonspecific binding (positive control), and a final test for buffer (negative control). As the identity tests are "pass/fail" tests, there is no need for quantitative limits for these tests. These tests are also adaptable to testing of starting materials for confirmation of identity for release testing. In addition to characterization of binding affinity for antigen, certain other types of tests might be used to establish the identity of a monoclonal antibody. These include peptide mapping, mass spectrometry, and SDS-PAGE.

17.8.2 Potency

For a monoclonal antibody, the most commonly employed methods for determining potency (assay) are protein determination by measurement of ultraviolet absorbance at 280 nm and binding efficacy for the specific antigen determined with ELISA. The binding efficacy of a sample determined using ELISA is typically compared with that of the reference standard. The label claim is usually expressed in units of mg/ml and/or mg per vial, and the limits around this value are typically fairly tight (±10% in this case). On the other hand, the fairly broad range associated with the potency determined by binding ELISA reflects the much higher variability associated with that assay.

17.8.3 Purity

At the DP level, purity assays for monoclonal antibodies typically are designed to examine a variety of product-related impurities, including size variants (heavy and light chain fragments, monomers, dimers, multimers, and other

Table 17.3 Drug product specifications for an early-phase monoclonal antibody product derived from CHO cells.

Attribute	Test	Acceptance criteria	Test method
Quality	Appearance	Clear, colorless to pale yellow solution, absence of visual particles	Visual
Quality	pH	5.8–6.2	USP <791>
Quality	Osmolality	270–330 mOsmol/kg	USP <785>
Quality	Volume in container	3.25–3.45 ml	USP <1>
Quality	Polysorbate 80	Report result	Gas chromatography
Potency	Protein concentration	9.0–11.0 mg/ml	UV spectroscopy
Potency	Binding ELISA	50–200%, relative to reference	ELISA
Identity	Binding ELISA	Comparable to reference	ELISA
Purity by SEC	Total low molecular weight peaks	≤2.00%	Size-exclusion chromatography
	Main peak	≥97.00%	
	Total high molecular weight peaks	≤1.00%	
	Report new peaks ≥0.05%, with relative retention times	Report result	
Purity by CEX	Acidic peaks	Comparable to standard; acidic peaks totaling 15.0 ± 5.0%	Cation-exchange chromatography
	Main peak	65.0 ± 15.0%	
	Basic peaks	Comparable to standard; basic peaks totaling 20.0 ± 7.0%	
Purity by SDS-PAGE	Reduced	Two major bands at approximately 25 and 50 kD, consistent with reference	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
	Nonreduced	One major band at approximately 150 kD, consistent with reference	
Microbial safety	Bacterial endotoxin	≤0.25 endotoxin units/mg	USP <85>
Microbial safety	Sterility	No growth	USP <71>

aggregates), charge variants, misfolded variants, and process-related impurities that might be of concern for the DP level. The size variants in this case are detected using size-exclusion chromatography and SDS-PAGE, while the charge variants are detected by cation-exchange chromatography (Figure 17.2). Most process-related impurities are controlled through specifications established at the DS level. For a monoclonal antibody produced in CHO cells, these might include host cell protein and DNA, as well as process solvents of concern (see Chapter 18 for a more in-depth discussion of the establishment of specifications for process-related impurities). Limits for the various purity assays should be established based on the capabilities of the analytical methods used, the batch history for the product, and the levels of exposure to the impurities in toxicology studies. The latter exercise requires close coordination with the nonclinical development team, as there are interspecies conversion factors to consider, as well as differences in dosing in amount, frequency, and duration.

17.8.4 Microbial Safety

Final product specifications for parenteral products must always contain endotoxin and sterility testing. Endotoxin limits are expressed in endotoxin units (EU) per mg, as in this case, or per ml. Endotoxin limits are established on the basis of route of administration (parenteral vs. intrathecal), patient body weight in kg, and the maximum dose (mg/kg) of drug to be administered over a 1 h period (see USP <85> [14] for a more detailed description of this calculation). To simplify the calculation, a typical patient body weight of 50 kg is often assumed. Specification limits for endotoxin are typically set that are well below the calculated endotoxin limit, especially if lower values can be routinely achieved. Sterility testing is a "pass/fail" test, so there is no need for a quantitative limit for this test.

17.8.5 Other Quality Attributes

A variety of basic product characterization assays are commonly lumped under the "quality" or "general characteristics" category. These may include visual appearance and visible particulate analysis, pH, osmolality (for parenteral drugs), and volume in container. Limits for pH are established not only on the basis of optimizing product stability but also to prevent injection site pain during administration to patients. Osmolality for a parenteral product should be roughly isotonic with blood plasma (the osmolality of human plasma is about 290 mOsmol/kg), and the limit shown in Table 17.3 is set accordingly. Volume in container is an important specification, as enough product must be able to be recovered from the primary container to satisfy the dose requirements, but on the other hand, too much overage might allow for overdosing of patients. In the present case a nominal dose of 3 ml is assured by the volume in container specification of 3.25–3.45 ml. For this monoclonal antibody DP, polysorbate 80

is used as an excipient added during formulation of the DP to protect the active ingredient against agitation and shear stress. The acceptance criterion of "report results" is specified since insufficient batch history exists at the present time to establish an acceptance range. Later in product development, this criterion will be changed to a quantitative range.

17.9 Case Study: Late-Stage Plasmid Product

Table 17.4 lists a set of specifications established for a hypothetical plasmid DS to be used to manufacture DP for a phase 3 clinical study. The rationale for inclusion of the various attributes and limits is discussed in the succeeding text, as are the justifications for changes made relative to an earlier version of the specification for this DS (also shown in Table 17.4).

17.9.1 Identity

For a plasmid product, restriction enzyme analysis is considered appropriate for a release test for a facility producing only one type of plasmid product, per the applicable guidance [15]. If at least one restriction enzyme does not make a cut within a unique region of the plasmid, a second method such as sequencing might be expected if the manufacturing facility were producing more than one plasmid product. An example of a unique plasmid region is the sequence coding for the product to be expressed in the patient.

17.9.2 Potency

Potency for a plasmid product is typically determined through measures of expression and bioactivity of the encoded gene [15]. In this case, potency is measured using a separate assay for all three attributes. A second assay (determination of ultraviolet absorbance at 260 nm) is used to determine nucleic acid concentration in the DS.

17.9.3 Purity and Product-Related Impurities

The specifications for circular forms and supercoiled forms of the plasmid were established to correspond with the manufacturing and stability experience to date. On stability, circular forms were always at least 98% of the total plasmid and supercoiled forms were 95% and above. FDA guidance [15] suggests that supercoiled forms should be ≥80% as an initial specification in the absence of manufacturing experience. Updating the specifications for circular forms and supercoiled forms prior to phase 3 manufacturing is therefore consistent with this guidance. However, the specification would not be applied retrospectively to batches already manufactured prior to the specification change.

Table 17.4 Evolution of DS specification for a plasmid product.

Attribute	Test	Early-stage acceptance criteria	Revised acceptance criteria
Quality	Appearance	Clear, colorless solution substantially free of visible particulates	Clear, colorless solution substantially free of visible particulates
Quality	pH	Report results	6.8–7.2
Quality	Osmolality	Report results	280–320 mOsmol/kg
Identity	Restriction analysis	Conforms to standard	Conforms to standard
Potency	Transfection[a]	None	>5 copies/cell
Potency	Expression	Expression 50–150%, relative to standard	50–150%, relative to standard
Potency	Bioactivity	None	Expressed product has 50–200% of the activity of product expressed by reference plasmid
Potency	Nucleic acid concentration by UV absorbance at 260 nm	≥3 mg/ml	≥3 mg/ml
Purity	A_{260}/A_{280} ratio	1.8–2.2	1.8–2.2
Purity	Total circular forms	≥90%	≥95.0%
Purity	Total supercoiled forms	Report value	≥90.0%
Impurities	Host cell RNA	<1%	<1.00%
Impurities	Host cell protein	<1%	<500 ppm
Impurities	Host cell DNA	<1%	<1.00%
Microbial safety	Bacterial endotoxin	<5 EU/mg	<54 EU/mg
Microbial safety	Total aerobic microbial count	<10 CFU ml	<10 CFU/100 ml
Microbial safety	Total yeast and mold count	<10 CFU/ml	<10 CFU/100 ml

[a] Transfection efficiency is required when a transfection agent, such as packaging in a noncompetent virus, is used or when the plasmid is delivered with a cationic polymer.

A measure of the relative content of nucleic acid in a sample versus the amount of protein may be obtained from the absorbance at 260 nm (nucleic acid max) versus protein (280 nm max). An A_{260}/A_{280} ratio greater than 1.0 reflects a relatively pure nucleic acid solution [16], and the specification has been established accordingly.

17.9.4 Process-Related Impurities

Residual host cell RNA, DNA, and protein criteria were updated to add additional precision. This is consistent with ICH Q3B(R2) [17] section 5, which indicates that impurities below 1.0% should be reported to two decimal places, while impurities above this level should be reported to one decimal place.

17.9.5 Microbial Safety

The endotoxin specification was adjusted to reflect the patient dose currently being used in the clinic. Further tightening of this specification might later be considered on the basis of additional manufacturing experience. The bioburden specification was tightened to correspond to the specification for water for injection. The previous specification was appropriate for early-stage manufacturing when sample volumes for qualification of the assay at this level were not available. The revised specification for bioburden was expected to be achievable since the bulk plasmid solution is filtered at 0.2 μm and filled into tubes under aseptic conditions.

17.9.6 Other Quality Attributes

Specifications for pH and osmolality were added since the formulation of the plasmid was changed from a formulation in water for injection to a formulation in phosphate-buffered saline. The formulation change was made in order to give patients an isotonic pH balanced dose with less pharmacy preparation. The specification of "report results" was appropriate for a non-buffered formulation, but not one in which excipients are added. The revised specifications were therefore intended to correspond to isotonic phosphate-buffered saline.

17.10 Conclusions

Specifications for biological products typically evolve during the course of product development as greater knowledge of critical product quality attributes, analytical method capability, and process consistency is gained. At early phases of development, for instance, a DP specification may have testing for impurities that specifies "report result" instead of an acceptance range or limit. Later in product development, the acceptance criterion may be changed to reflect an actual limit or acceptable range for the same impurities. Testing for specific process impurities may be removed during evolution of a DS or DP specification, if, for instance, it is demonstrated through process characterization/validation that consistent clearance of the given process impurity is

obtained. During the evolution of specifications for DS and DP, it should be kept in mind that removal of attributes (other than the specific case mentioned previously) and broadening of acceptance criteria for an attribute at the later stages of product development could result in added scrutiny from regulatory agencies. The expectations promulgated within ICH Q6B are that as product development progresses, specifications for the various product attributes will "be refined," with acceptance criteria potentially tightened and the justifications for each criterion substantiated with actual batch history data. These expectations are reasonable and should be achievable for a manufacturing process that is under control and is well characterized. An example of a specification for a plasmid product has been used to illustrate the evolution from early phase to later phase.

References

1 International Conference on Harmonisation (ICH) 1999. Q6B. Specifications: Test procedures and acceptance criteria for biotechnological/biological products.
2 United States, Food and Drug Administration (US FDA) 2015. General principles of IND submission. Code of Federal Regulations, Title 21, Part 312.23.
3 European Medicines Agency 2012. Guideline on the requirements for quality documentation concerning biological investigational medicinal products in clinical trials. EMA/CHMP/BWP/534898/2008.
4 United States, Food and Drug Administration (US FDA) 1995. Guidance for Industry—Content and format of Investigational New Drug Applications (INDs) for phase 1 studies for drugs, including well-characterized, therapeutic, biotechnology-derived products. Center for Biologics Evaluation and Research.
5 United States, Food and Drug Administration (US FDA) 2008. Guidance for FDA reviewers and sponsors: Content and review of chemistry, manufacturing, and control (CMC) information for human gene therapy Investigational New Drug Applications (INDs). Center for Biologics Evaluation and Research.
6 United States, Food and Drug Administration (US FDA) 2003. Guidance for Industry: INDs for phase 2 and phase 3 studies—Chemistry, manufacturing, and controls information. Center for Drug Evaluation and Research.
7 Rao A. 2014. Regulatory perspectives on setting relevant specifications in early development and throughout product life cycle.
8 International Conference on Harmonisation (ICH) 2009. Q8(R2). Pharmaceutical development.
9 International Conference on Harmonisation (ICH) 2005. Q9. Quality risk management.

10 International Conference on Harmonisation (ICH) 1995. Q5. Stability testing of biotechnological/biological products.
11 United States Pharmacopeia <1226> Verification of compendial procedures.
12 United States, Food and Drug Administration (US FDA) 2004. Guidance for Industry PAT—A framework for innovative pharmaceutical development, manufacturing, and quality assurance. Center for Drug Evaluation and Research, Center for Veterinary Medicine, and Office of Regulatory Affairs.
13 Woodcock J. 2004. The concept of pharmaceutical quality. *Am Pharm Rev* 7:10–15.
14 United States Pharmacopeia <85> Bacterial endotoxins test.
15 United States, Food and Drug Administration (US FDA) 2007. Guidance for Industry. Considerations for plasmid DNA vaccines for infectious disease indications.
16 Held P. 2008. Measure your purity. Assessment of nucleic acid purity via UV absorbance. *GIT Lab J* 5:9–11.
17 International Conference on Harmonisation (ICH) 2006. Q3B(R2). Impurities in new drug products.

18

Process-Related Impurities in Biopharmaceuticals

A Deeper Dive into ICH Q6B

*Anil Raghani[1], Kim Li[2], Jeanine L. Bussiere[2], Joel P. Bercu[3], and Jinshu Qiu[2]**

** Corresponding author*
[1] Coherus BioSciences, Inc., Camarillo, CA, USA
[2] Amgen Inc., Thousand Oaks, CA, USA
[3] Gilead Sciences, Inc., Foster City, CA, USA

18.1 Introduction

International Conference on Harmonisation Q6B [1] provides guidance on the general principles for setting release and in-process specifications for biopharmaceuticals and for justifying the specifications established for product- and process-related impurities. The intent of the guidance is to ensure the quality, safety, and efficacy of these drugs when delivered to patients. The Q6B guidance describes two types of impurities: product- and process-related. Product-related impurities are molecular variants of the biopharmaceutical, which may not possess the same activity, efficacy, and safety as the active molecule. Examples of product-related impurities are truncated and posttranslational modifications and aggregates. Process-related impurities, on the other hand, are those that are derived from the manufacturing process such as cell substrates, cell culture and fermentation media components, and downstream process-derived impurities.

Establishment of product release, in-process, and product-related impurity specifications is addressed in Chapter 17. The purpose of this chapter is to describe in more detail procedures that may be used for setting safety limits and analytical strategies for process-related impurities, specifically process reagents, in biopharmaceuticals to ensure compliance with Q6B.

The principles and strategies underlying establishment of specifications for process-related impurities in biopharmaceuticals have been adopted from similar guidance documents applicable to chemically derived drug substances (DS).

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For instance, ICH Q3A(R2) [2] and Q3B(R2) [3] (Chapter 6) provide the framework for the reporting, identification, and qualification of impurities in DS and drug products (DP), respectively. Impurities are classified in these guidance documents as organic impurities (process- and drug-related), inorganic impurities, and residual solvents. More details on deriving limits for different types of process impurities are described in additional ICH guidance documents, such as Q3C(R5) [4] (Chapter 7) for residual solvents, Q3D [5] (Chapter 8) for elemental impurities, and M7 [6] for mutagenic impurities.

In this chapter, examples will be provided to illustrate cases of the interrelationship between ICH Q3A(R2) and Q6B for the risk assessment of process-related impurities arising from biopharmaceutical manufacturing processes.

18.2 Controlling Process-Related Impurities

A large number of process reagents (a term for raw materials) are used in the manufacture of biopharmaceutical products, and these are major potential sources of process-related impurities. One of the strategies for mitigating risks associated with process-related impurities is to test for the clearance of process reagents in downstream purification steps. Minimizing unnecessary clearance testing of process reagents, while ensuring product quality and safety, is a key element of any control strategy. This is in part achieved through appropriate classification of reagents. On the basis of their safety profiles and histories of safe use, process reagents may be classified into two categories: generally recognized as safe (GRAS) and potential safety concern (PSC) reagents [7]. Reagents considered GRAS are excipients that have acceptable uses in commercial parenteral DP [8–10] and in parenteral nutritional therapy [11], trace elements that have parenteral permissible daily exposure (PDE) limits [5], and inactive ingredients of parenteral products listed in the US Food and Drug Administration (FDA) database [12]. Therefore, the GRAS term used in this chapter has a narrower definition compared with the term "generally regarded as safe" defined in the Code of Federal Regulations [13]. Any reagent not regarded as GRAS is classified as a potential safety concern reagent.

Two terms are frequently used in this chapter: *clearance testing* and *specification testing*. The former refers to the process reagent testing required for a product during its process development, process characterization, and process validation stages, and the latter refers to the testing needed for lot release during commercial production of a product. Requirements for clearance testing and associated specification testing for process reagents can be determined through consideration of four risk factors [7]: (i) the safety profile of the reagents, (ii) the starting amount of the reagents used in the manufacturing process, (iii) the maximum dose of the product and the amount of DS required to produce that dose, and (iv) the point of introduction of the reagents in the overall manufacturing process.

Generally, clearance testing of GRAS reagents is not required because of the safety profiles and historically safe use of these reagents, as well as the capability of manufacturing processes to remove them. Evaluation of GRAS reagents that are introduced at high concentration during late downstream purification steps (i.e., steps that are not followed by additional purification processes) should be carefully considered and where necessary analyzed to ensure that the levels are acceptable in terms of both safety and quality. For PSC reagents, the four risk factors described previously must be evaluated. In the following section, a general procedure for evaluating clearance testing requirements is described. The process for establishing the need for specification testing for a PSC reagent includes deriving a PDE limit, impurity and control strategy assessment, process evaluation, safety margin calculation, and risk assessment employing failure modes and effects analysis (FMEA).

18.3 General Methodology for Deriving Limits for Process-Related Impurities

Adequate clearance of a process reagent occurs when the concentration is reduced through upstream and downstream processes to a level that is considered acceptable. The acceptability of a process reagent concentration remaining in a DS is determined on the basis both of its concentration and the potential toxicological impact on patients receiving the associated DP. To determine the appropriate toxicology-based limit for a process reagent in a DS or DP, a PDE or acceptable intake (AI) is derived. The PDE or AI, the maximal acceptable exposure per day of an impurity in a pharmaceutical [4–6], is used to develop a health-based protective limit for patients exposed to the residual impurity. The PDE or AI is derived by analyzing all potential toxic effects, such as acute, chronic, reproductive toxicity and carcinogenicity in both animals and humans. From this information, the most sensitive effect is applied to the assessment.

A methodology for deriving a PDE for residual solvents is described in Q3C(R5) [4]. This is a standardized approach with transparent methods for selecting the toxicology studies, critical effects, and modifying factors to be considered. Because solvents are completely bioavailable, a PDE developed per Q3C(R5) is typically applicable for all routes of exposure. A similar methodology has also been applied in Q3D [5] to inorganic elements and metals, with an additional consideration for different routes of exposure such as oral, parenteral, or inhalation.

The majority of process-related impurities in biopharmaceuticals are low molecular weight organic and inorganic compounds. The methodologies described in Q3C(R5) and Q3D are therefore also considered applicable when generating limits for chemically based reagents in biopharmaceuticals.

For noncarcinogenic effects, the following PDE calculation is applied:

$$\text{PDE} = \frac{\text{NOEL or LOEL} \times \text{body weight } (\text{kg})}{\text{Modifying factors} \times \alpha}$$

where the body weight adjustment can range from 50 to 70 kg
NOEL—no observed effect level
LOEL—lowest observed effect level

Note: The NOEL or LOEL is the most sensitive effect (lowest dose) that is relevant to humans for noncarcinogenic effects such as acute, chronic, reproductive, and developmental toxicity. Human data are preferred if available. Often, toxicology studies report the no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL), and one of these may be applied in the PDE calculation instead of the NOEL or LOEL.

And the modifying factors include the following:

1) F1 = Interspecies extrapolation
   a) F1 = 5 for extrapolation from rats to humans
   b) F1 = 12 for extrapolation from mice to humans
   c) F1 = 2 for extrapolation from dogs to humans
   d) F1 = 2.5 for extrapolation from rabbits to humans
   e) F1 = 3 for extrapolation from monkeys to humans
   f) F1 = 10 for extrapolation from other animals to humans
2) F2 = Interindividual variability
   a) A factor of 10 to account for variability between individuals
3) F3 = Duration of exposure
   a) F3 = 1 for studies that last at least half of a lifetime (1 year for rodents or rabbits)
   b) F3 = 1 for reproductive studies in which the whole period of organogenesis is covered
   c) F3 = 2 for a 6-month study in rodents or a 3.5-year study in non-rodents
   d) F3 = 5 for a 3-month study in rodents or a 2-year study in non-rodents
   e) F3 = 10 for studies of shorter duration
4) F4 = Nature of toxicity
   a) A factor that may be applied in cases of severe toxicity, for example, nongenotoxic carcinogenicity, neurotoxicity, or teratogenicity
   b) In studies of reproductive toxicity, the following factors are used:
      i) F4 = 1 for fetal toxicity associated with maternal toxicity
      ii) F4 = 5 for fetal toxicity without maternal toxicity
      iii) F4 = 5 for a teratogenic effect with maternal toxicity
      iv) F4 = 10 for a teratogenic effect without maternal toxicity

5) F5 = Quality of data
   a) A variable factor that may be applied if the NOEL was not established. When only an LOEL is available, a factor of up to 10 might be used, depending on the severity of the effect observed.
6) α = Bioavailability adjustment
   a) Large molecules are likely administered parenterally, such as by an intravenous (IV) or subcutaneous (SC) route. If a reagent under consideration has lower oral bioavailability, a bioavailability adjustment should be used when extrapolating from oral data. When only oral data or data from other routes are available, the bioavailability of the given route is used to extrapolate to a parenteral limit.

For carcinogenic effects, it is assumed that there is no threshold and any dose can result in a carcinogenic response. An AI is the dose that results in a negligible excess risk of cancer over background [6]. Per ICH M7, the acceptable excess cancer risk for carcinogenic impurities is one in 100,000. Calculation of the AI is based on linear extrapolation from the biologically effective dose in animals. In cases where specific carcinogenic data are available (e.g., from the Carcinogenicity Potency Database) [14], these data, typically in the form of a TD_{50} value, should be used to establish a compound-specific limit. For process reagents that are mutagenic (i.e., positive in the Ames mutagenesis assay) and for which there are no specific carcinogenicity data, the threshold of toxicological concern (TTC) is used to derive the AI. The TTC is the threshold (e.g., 1.5 μg/day, daily for lifetime) that is considered unlikely to exceed one in 100,000 excess risk of cancer when the dose–response relationship of the mutagenic compound is unknown [15, 16].

While the method described previously uses a linear approach for carcinogenicity, there are several examples where a carcinogenic endpoint is not appropriately evaluated using AI or TTC methodology. For instance, when a carcinogen acts via a non-mutagenic mechanism and a threshold can be demonstrated, the PDE methodology is applied [6]. For example, a threshold approach can be used based on splenic tumors observed in rats, since these have a nonlinear dose–response, and mutagenicity/genotoxicity is not central to the mode of action of aniline-induced carcinogenicity [17]. The PDE is derived using the process defined in Q3C(R5). Finally, there are mechanisms of carcinogenesis that are specific to certain nonhuman animal species/strains, and these animal models are not considered relevant to humans. In such cases, no PDE or AI is developed on the basis of these carcinogenicity data, and a PDE is developed based on other types of toxicity data (e.g., reproductive or chronic toxicology studies).

Some process reagents have a limited toxicology data set and there may not be enough data to derive a PDE or AI. In such cases, the "process of acquiring and evaluating data that establishes the biological safety of an individual

impurity or a given impurity profile at the level(s) specified" [4] described in Q3A(R2) should be applied. The qualification limit for the process reagent for a DS dose that is ≤2 g/day is 0.15% or 1 mg/day, whichever is lower. The qualification limit is 0.05% for a DS dose that is >2 g/day. If a process reagent is to be used above this qualification limit, it should be tested for toxicity in accordance with Q3A(R2). Alternatively, consideration could be given to reducing the impurity level below the qualification limit through manufacturing processes.

The Q3A(R2) guideline highlights that lower thresholds should be set if the process impurity is unusually toxic. An example of an unusually toxic process reagent is one that is mutagenic and/or carcinogenic. For such impurities, the toxicological principles described in ICH M7 for deriving limits (i.e., AI) may be applied as these are typically low molecular weight impurities. There may be toxic effects other than mutagenicity and carcinogenicity that result in a process impurity being considered a PSC but for which insufficient data exist to allow calculation of a PDE. Such cases are handled on a case-by-case basis, and the TTC can be applied to account for such non-mutagenic effects to derive a PDE [16, 18].

Once a PDE or AI is calculated for a process reagent, an exposure limit (L_e) can be derived for the DS or DP. The L_e is calculated by dividing the PDE or AI of a process reagent by the maximum daily dose of the DP. The units for L_e are therefore μg/dose. The L_e is a critical parameter for determining the safety margin of a process reagent.

In most cases, adjustments are not made for specific pharmaceuticals or patient populations in determining the PDE or AI. This allows a conservative PDE or AI to be applied for different DS types. However, there are certain instances where the toxicology-based qualification limit cannot be met due to process limitations or limitations in sensitivity of analytical methods applicable to the impurity. In such cases, the PDE or AI may be adjusted based on knowledge of the DP, its indication, and the patient population. This leads to generation of a product-specific PDE or AI. For example, a patient population (i.e., oncology) might warrant a higher product-specific PDE or AI limit. The phase of development (early clinical trials vs. marketed drug), adjustment for bioavailability, qualification of levels in toxicology studies, or duration of exposure/dosing interval (e.g., once weekly for 3 months, once monthly, once every six months, etc.) might also warrant a product-specific PDE [19].

18.4 Assessment of Impurities and Control Strategies

Process-related impurities can affect the safety and efficacy of a DP. The attributes of a DP considered essential to ensure safety and efficacy are referred to as critical quality attributes (CQAs) per ICH Q6B. The process-related impurities identified as a CQA of a DP must be below specified limits.

Several strategies may be adopted to control process-related impurities produced in bioprocesses. One approach involves following the quality by design (QbD) principles described in ICH Q8(R2) [20]. The interdependence of process reagent quality, process performance, and product quality is often assessed during process development. In cases where there is a strong correlation between the quality of a process reagent and product quality, tightly controlling the quality of such a process reagent is an acceptable strategy to control CQAs. The other control strategy often employed by the manufacturer is to test for the clearance (removal) of PSC reagents at one or more unit operations. When a PSC reagent is identified as a CQA, a specification must be developed for the reagent in the DS or DP so that the determined limit is not exceeded during manufacture of the DS or DP.

18.5 Evaluation of Process Capability and Capacity for Removing Impurities

In order to ensure that specification testing is focused on those parameters and attributes considered critical, biopharmaceutical manufacturers may adopt a risk-based approach to demonstrate adequate clearance of a PSC reagent and to preclude the need for specification testing. A good understanding of both impurity properties and the manufacturing processes is ultimately key to the conduct of the risk assessment required to justify the elimination of specification testing for an impurity. The following are some of the factors considered in the risk-based approach to minimize specification testing for a PSC reagent:

- The process capability for clearance of the impurity
- The process capacity for clearance of the impurity
- Process performance consistency for clearance of the impurity
- Potential process changes
- Scale-up impacts on clearance of the impurity
- Starting concentration of the PSC reagent observed in the process stream relative to the determined toxicology-based exposure limits

Understanding of the capability of a process to remove process-related impurities is essential for risk-based assessment to determine whether specification testing is required. A PSC reagent removed upstream of the purification steps will be generally less likely to pose a risk in comparison with one removed downstream or near the end of the process flow, since the upstream provides more process capability to remove PSC reagents through additional clearance and purification steps than later downstream. Similarly, physicochemical properties and chemical structure of a PSC reagent play significant roles in the risk assessment exercise. If a column purification step involves a high degree of

specificity for a biopharmaceutical molecule and the PSC reagent has vastly different physicochemical or structural properties relative to the biopharmaceutical molecule, it is more likely that the PSC reagent will be effectively separated from the product.

The amount of process-related impurities removed as a result of each unit operation is also critical in the evaluation of the specification testing requirement. When a process step has a large capacity to remove PSC reagents, it is unlikely that those PSC reagents will be present in the DP above their determined exposure limits. This is especially true when that unit operation is followed by other operations that may contribute to clearance of the impurity. During the process design phase, it is therefore important to determine the process capability and capacity to remove PSC reagents so that the need for the specification testing for PSC reagents can be determined.

When the scale of a process changes, it may be necessary to conduct a comparison of process capability and capacity to remove process-related impurities at each scale. If a small-scale model is well qualified, and data for the removal of PSC reagents at the small scale are available, the testing for such PSC reagents at the larger scale may be unnecessary. This argument is particularly applicable when the process has large excess capacity to remove the PSC reagents. In such cases, changes occurring during scale-up are unlikely to significantly impact the capacity of the process to remove PSC reagents, and hence this should negate the need for any specification testing.

In addition to having an in-depth understanding of process capability and capacity for the removal of process-related impurities, knowledge of the concentrations of such impurities expected to occur as process reagents or by-products of the process relative to their known toxicities is essential for the risk-based approach to determine the need for specification testing. For instance, if the concentration of a PSC reagent present in the process stream is much lower than its exposure limit, assuming the entire amount of the PSC reagent is co-purified with the product through downstream processing, it may not be necessary to perform specification testing for this reagent.

18.6 Safety Margin and Determining the Need for Clearance and Specification Testing

The safety margin (S) is defined as the magnitude of the difference between the exposure limit (L_e) of a PSC reagent a patient may be safely exposed to on a daily basis without a long-term toxic effect and its maximum level present in a product dose (M). It is expressed as a ratio of the exposure limit to the maximum reagent level in a product dose, $S = L_e/M$ [7].

The safety margin combines three critical factors—(i) reagent toxicity, (ii) reagent amount, and (iii) drug dose into a single calculation—and provides a unit-less numeric value to measure the magnitude of reagent safety. The safety margin indicates that if the maximum amount of a process reagent present in a product dose is equal to the exposure limit, S is equal to 1. The safety margin $S = 1$ is a critical value that can be used as a criterion for determining reagent safety. When $S \geq 1$, the maximum amount of a reagent present in a product dose is equal to or less than the exposure limit and the reagent will not pose a safety concern. When $S < 1$, the amount of a reagent present in the product is too high and may adversely impact patient safety. Therefore, the safety margin can be used as a science-based criterion for determining the need for clearance testing and specification testing.

Depending on the source of a PSC reagent used in the calculation, the safety margin can be divided into estimated safety margin (S_e) and measured safety margin (S_m). The value S_e can be used for determining the need for clearance testing at the process development phase, while S_m can be used for determining the need for clearance and specification testing at the late phase and commercial production stage.

18.6.1 Calculation of Estimated Safety Margin

An estimated safety margin (S_e) is calculated as shown in Equation 18.1:

$$S_e = \frac{L_e}{M_e} \tag{18.1}$$

where L_e is the exposure limit for a PSC reagent in μg/dose and M_e is an estimated maximum amount of the PSC reagent per product dose in μg/dose. The value M_e is calculated by dividing the total amount of reagent added to a bioreactor by the total amount of DS produced and then normalizing this ratio to a DS dose, assuming no reagent clearance occurs throughout the entire purification process. For example, if total amount of a PSC reagent added is 0.12 g/l (or 120,000 μg/l), total amount of DS produced is 3.1 g/l (or 3,100,000 μg/l), and a drug dose is 800,000 μg, then M_e is calculated as

$$M_e = \frac{120{,}000\,\mu g/l}{3{,}100{,}000\,\mu g/l} \times 800{,}000\,\mu g/\text{dose} = 30{,}968\,\mu g/\text{dose}.$$

When $S_e < 1$, the estimated amount of a PSC reagent per DS dose is higher than its exposure limit, and clearance testing of the PSC reagent is necessary at the process development phase. When S_e is ≥1, the estimated amount of a PSC reagent per DS dose is lower than its exposure limit, and clearance testing of the PSC reagent is not required at any stage of development.

18.6.2 Calculation of Measured Safety Margin

A measured safety margin (S_m) is calculated as shown in Equation 18.2:

$$S_m = \frac{L_e}{M_m} \tag{18.2}$$

where M_m is the measured maximum amount of the reagent per product dose in μg/dose and L_e is the exposure limit for a PSC reagent in μg/dose. When S_m is <1, either the process reagent should be changed or the manufacturing process may be modified to ensure $S_m \geq 1$ prior to use of the product in humans; additional testing in animal species could provide data to revise the L_e, or a revised L_e may be appropriate based on the specific patient population and/or frequency of dosing (i.e., on the basis of a product-specific PDE or AI). On the other hand, if S_m is high enough such that moderate fluctuations observed in the process will not result in the amount of the PSC reagent in the product becoming close to its exposure limit, specification testing for the PSC reagent in the product may not be required. For example, if the S_m value of a PSC reagent is 10 with an exposure limit of 40 μg/dose at the target process parameters, and if the lot-to-lot variation in the measured amount of that reagent is 4 ± 2 μg/dose, the S_m values for these lots would vary from 6.7 to 20 from lot to lot. This range is wide enough to justify that such a well-controlled process does not need to be monitored by specification testing for the removal of the process reagent from each lot produced using such a process. If a S_m is low (1–5), it could become <1 for any given lot due to large lot-to-lot variability. In this case, regulatory agencies may require specification testing of the PSC reagent in each lot as a release assay. On the other hand, a high S_m (≥5) provides a reasonable justification that testing is not required.

A graphic illustration of the use of S_e and S_m to determine the need for clearance and specification testing at different stages in the product development life cycle is shown in Figure 18.1 [7].

18.7 Conduct of the Risk Assessment for Elimination of Specification Testing

Many biotechnology companies take a platform approach to the manufacture of biopharmaceuticals. The platform approach involves using a set of previously developed process operating conditions for manufacturing a new molecule. Application of platform processes allows the manufacturer to advance a new molecule within the product life cycle more quickly. Because of extensive experience gained through the application of the same process operating conditions multiple times for various molecules, platform processes become very well understood. This experience is beneficial in determining the clearance testing

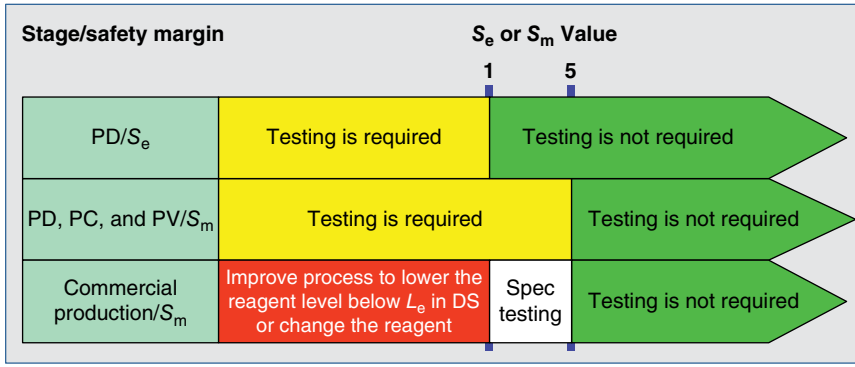


Figure 18.1 Safety margin (S_e or S_m) as a criterion to determine the requirement for clearance and specification testing for PSC reagents at process development (PD), process characterization (PC), process validation (PV), and commercial production stages.

requirements of process reagents. If a manufacturer has demonstrated the removal of process reagents from a given platform process, it could be argued that further demonstration of process reagent clearance by the same platform process applied to a different molecule should not be required. In such cases, regulatory agencies may accept these arguments, allowing a manufacturer to eliminate reagent clearance testing at the early stage of the process development. This is particularly true when the operating parameters of the platform process are wide enough and the process parameters in question fall within that operating space.

The need for specification testing for a process reagent should be further assessed on the basis of in-depth risk assessment following a procedure such as a FMEA. The three critical components of FMEA are assessment of (i) severity of the effect (i.e., toxicity), (ii) occurrence of the effect, and (iii) detectability of the effect [21]. A total risk score derived from these three components provides an indication of the overall risk associated with a given failure effect attributed to a process reagent.

18.7.1 Estimated Safety Margin as a Measure of Severity

A comparison of the total amount of a PSC reagent in a process stream compared with its toxicology-based exposure limit provides a S_e. This S_e provides an indication of the severity of the risk that must be taken into account in the FMEA establishing the specification testing requirement. Quantification of a PSC reagent at each unit operation of the purification provides an understanding not only of the points of the manufacturing process where the PSC reagent may be removed but also of the capacity of these operations to remove the PSC reagent. The reciprocal of the estimated safety margin ($1/S_e$) provides guidance

on the minimum clearance required by the overall process to reach the safe level of a PSC reagent.

18.7.2 Fold Clearance as a Measure of Occurrence

The capacity to remove impurities from a process can be measured by calculating fold clearance. Fold clearance is the amount of a PSC reagent removed from the process when its amount is determined at the start and the end of the process. Fold clearance data can be used as a measure of occurrence when conducting the FMEA. Establishment of fold clearance is typically achieved during the process development phase and during validation of the process in question. When the fold clearance shown for a given impurity during the process development phase is orders of magnitude higher relative to the minimum clearance required for the product to be considered safe, specification testing for that impurity during the release of each lot may not be necessary. It is therefore important to gather fold clearance data for multiple product lots in order to justify elimination of specification testing for PSC reagents.

18.7.3 Measured Safety Margin as a Measure of Detectability

It may be the case that certain PSC reagents are not completely removed from the process, resulting in a residual amount of the PSC reagent in the product. After clearance testing has been conducted, S_m should be calculated for any PSC reagent with an S_e value of <1. Clearance testing at the process characterization and validation stages should be conducted if S_m is between 1 and 5. Specification testing for process reagents is warranted when the measured amount of process reagents in the product is close to the exposure limits for the process reagents (S_m is between 1 and 5), particularly when there is a large variation in the amount of process reagent observed in the product from manufactured lot to lot. Therefore, S_m serves as a good measure of detectability in the FMEA risk assessment procedure.

18.7.4 Interpreting the FMEA

The overall risk of a process impurity can be quantified by multiplying scores associated with severity, occurrence, and detectability. As discussed previously, these risk factors are determined from the estimated safety margin, fold clearance, and the amount of a PSC reagent observed in the product. A general guidance for interpreting the scores for severity, occurrence, and detectability is provided in Table 18.1. The need for risk mitigation is ordinarily determined on the basis of overall risk scores. A higher score (e.g., an overall score of 9 or greater in Table 18.1) would mean more rigorous controls and a need for specification testing of that particular PSC reagent.

Table 18.1 Examples of scoring of severity, occurrence, and detectability during a risk assessment to determine the need for specification testing.

FMEA component	Performance parameter	Range	Score	Interpretation
Severity	S_e (theoretically estimated safety margin)	0.0–1.0	9	Need specification testing
		>1.0	1	No specification testing is required
Occurrence	Fold clearance	0.0–1.0	9	Improve process capability or implement specification testing
		1.1–4.9	7	Specification testing may be required
		5.0–10.0	3	Process has acceptable capacity to remove impurities; no specification testing is required
		>10.0	1	Process has excess capacity to remove impurities; no specification testing is required
Detectability	S_m (measured safety margin)	0.0–1.0	9	Improve process capability
		1.1–4.9	7	Specification testing may be required
		5.0–10.0	3	No specification testing is required
		>10.0	1	No specification testing is required

18.8 Case Studies of Interrelationship between Impurity, Risk Assessment, and Need for Specification Testing

Since a large number of process reagents are used in biopharmaceutical manufacturing, the procedure described in this chapter should be employed for biopharmaceutical products during the manufacturing process development stage so that clearance testing or specification testing can be focused on PSC reagents, sometimes including a few GRAS reagents that may impact product quality and pose a safety concern if their levels present in DS or DP are high. General processes for evaluating process reagents, process capability, specification testing, and potential risk are discussed in the previous sections. The case studies described in the succeeding text use hypothetical data, and the

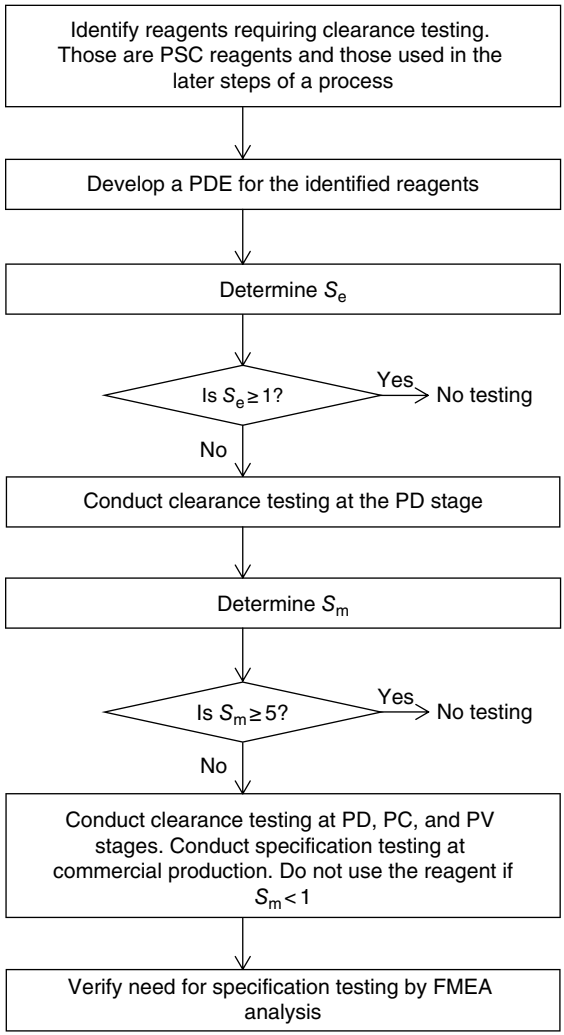

Identify reagents requiring clearance testing. Those are PSC reagents and those used in the later steps of a process
Develop a PDE for the identified reagents
Determine S_e
Is $S_e \geq 1$?
Yes
No testing
No
Conduct clearance testing at the PD stage
Determine S_m
Is $S_m \geq 5$?
Yes
No testing
No
Conduct clearance testing at PD, PC, and PV stages. Conduct specification testing at commercial production. Do not use the reagent if $S_m < 1$
Verify need for specification testing by FMEA analysis

Figure 18.2 Procedure for determining the need for reagent clearance and specification testing at the PD, PC, PV, and commercial production stages.

purpose of the case studies is to provide a detailed procedure for conducting these processes. The workflow of the procedure is illustrated in Figure 18.2.

18.8.1 Developing a PDE

In the following section the steps taken to develop toxicology-based PDE values for 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (CAS 7365-45-9), 3-N-morpholinopropanesulfonic acid (MOPS) (CAS 1132-61-2), methotrexate (MTX) (CAS 59-05-2), and Pluronic F68 (Poloxamer 188; CAS

Table 18.2 Toxicology-based permissible daily exposures (PDEs) for process reagents.

		Modifying factors						
Reagent	**Critical effect**	**F1**	**F2**	**F3**	**F4**	**F5**	**α**	**PDE (μg/day)**
HEPES	Not enough data to derive a PDE. Use ICH Q3A limits	—	—	—	—	—	—	NA
MOPS	Not enough data to derive a PDE. Use ICH Q3A limits	—	—	—	—	—	—	NA
MTX	Lowest clinical dose for rheumatoid arthritis (0.125 mg/kg/week) is averaged to 0.018 mg/kg/day	1	5	10	10	2	1	1.1
Pluronic F68	NOAEL of 20 mg/kg/day in a 3-month SC dog study	2	10	5	1	1	1	12,000
	NOAEL of 10 mg/kg/day in a teratology SC rat study	5	10	1	1	1	1	12,000

9003-11-6) are described. For HEPES and MOPS, a thorough literature search did not reveal sufficient toxicology data to derive a PDE (Table 18.2). Both HEPES and MOPS are buffers commonly used for mammalian cell culture at concentrations up to 10–25 mM and 20 mM, respectively, and are considered to be nontoxic at these concentrations. HEPES is administered as a buffering inactive ingredient in the IV diagnostic radiopharmaceutical indium oxyquinoline (6 mg/ml) [22]. These data indicate that HEPES and MOPS are of a low order of toxicity, but the data by themselves are not sufficient to allow calculation of a PDE. These process impurities were therefore not considered "unusually toxic" and limits were generated using per ICH Q3A(R2).

MTX has been in clinical use for over 50 years, with an extensive clinical and nonclinical data set. MTX inhibits dihydrofolic acid reductase and is administered for the treatment of certain neoplastic diseases, severe psoriasis, and adult rheumatoid arthritis. It may be given by the intramuscular (IM), IV, intra-arterial, or intrathecal routes. It is a well-known developmental/reproductive toxicant and is classified as a pregnancy category X drug [23]. All relevant nonclinical and clinical endpoints were considered, and the critical toxicological effect selected for use was the low dose for rheumatoid arthritis, which was 7.5 mg/week or 1.1 mg/day when averaging the single dose over 7 days [23, 24]. A total cumulative modifying factor of 1000 was applied to the daily dose to derive a PDE. Nontraditional safety factors were applied in certain cases to reflect that the critical effect was based on a sensitive endpoint observed in clinical trials with minimal adverse effects [25]. The PDE expressed in mg/kg body weight was also over 5000-fold less than the lowest dose shown to result in developmental toxicity in rats (0.1 mg/kg by intraperitoneal injection).

Pluronic F68 is present as an inactive ingredient in US FDA-approved pharmaceutical products administered by oral, topical, ophthalmic, SC, and IV routes [12]. Both clinical and nonclinical data are available for Pluronic F68. There were two critical effects, which both resulted in the same PDE. The first critical effect was a NOAEL of 20 mg/kg/day observed in a subchronic (3 months) SC dog study, which was the highest dose tested. The second critical effect was a NOAEL of 10 mg/kg/day observed in a rat SC teratology study. Mild increases in hemorrhages of the head and within the abdomen were observed at 100 mg/kg/day and above [26]. A total cumulative modifying factor of 50–100 was applied to the daily dose from the two studies to derive the PDE values (Table 18.2).

The PDE values shown in Table 18.2 are expressed in units of μg/day or μg/week. Since a drug is dosed daily or weekly, a L_e value in μg/dose is obtained by dividing the PDE value by dose/day or dose/week. The L_e values for the four reagents are shown in Table 18.2.

18.8.2 Estimated Safety Margin and Clearance Testing

The requirement for testing the removal of a process-related impurity can be determined based on the S_e (Equation 18.1). The case studies provided in this section use hypothetical examples of the amounts of process reagents and the process conditions.

MTX is used in mammalian cell culture processes as a selection agent. The dose-normalized amount is 0.04 μg/dose. Even assuming that the entire amount of MTX co-purifies with the product, the amount of MTX used in this process is far less than its exposure limit of 1.1 μg/dose. This leads to an estimated safety margin, S_e, of 27.5. In this case, no clearance testing is necessary. The assumption of the co-purification of the process reagent is a worst-case assumption, because process impurities such as MTX are typically expected to be removed through the purification process. The estimated safety margin of 27.5 provides significant tolerance in case the process is not tightly controlled.

The risk assessment applied to Pluronic F68 provides a similar result (Table 18.3), given that this process reagent yields a S_e above 6. However, because S_e is determined to be <1.0 for both HEPES and MOPS, clearance testing for these reagents in the purified product becomes necessary (Table 18.3).

18.8.3 Measured Safety Margin and Specification Setting

A hypothetical process uses 67.6 mg/dose of HEPES to control the pH of the cell culture during the production. The exposure limit of HEPES is the ICH Q3A limit of 1 mg/dose, which yields an estimated safety margin of 0.01 (Table 18.4). The process must be able to remove 1/0.01 (100-fold) HEPES in order to ensure that the impurity is below the safe level in the DP. Because the

Table 18.3 Determining the need for clearance testing.

	Process reagent			
Risk factors	**Methotrexate**	**Pluronic F68**	**HEPES**	**MOPS**
Unit operation reagent is introduced	N-3 cell bag	Production bioreactor	Production bioreactor	Third purification column
Estimated maximum amount of reagent in DS in the absence of clearance (μg/dose)	0.04	1,975	67,601	3760
Estimated exposure limit, L_e (μg/dose)	1.1	12,000	1,000	1000
Estimated safety margin, S_e	27.5	6.1	0.01	0.3
Testing required ($S_e < 1.0$)	No	No	Yes	Yes
Minimum process clearance required to not exceed exposure limit (fold)	None	None	100	3.7

Table 18.4 Hypothetical process capacity to remove process-related impurities.

	Process reagent	
Risk factors	**HEPES**	**MOPS**
Estimated maximum amount of reagent in DS in the absence of clearance (μg/dose)	67,601	3760
Estimated exposure limit, L_e (μg/day)	1,000	1000
Minimum process clearance required to not exceed exposure limit (fold)	67.6	3.8
Amount of reagent observed in DS (μg/dose)	<1.28	860
Actual fold clearance achieved	52,813	4.4
Measured safety margin, S_m	781	1.2

estimated safety margin is <1.0, this process-related impurity must be tested for its removal at the process development phase. The amount of this impurity in the final product is below the limit of quantitation of 1.28μg/dose (Table 18.4), and this indicates that the process has sufficient capacity to remove HEPES, giving 52,813-fold clearance when compared with the starting amount of this

reagent used in the process. As a result, the measured safety margin (S_m) is calculated to be 781. Since the S_m is much higher than 5, no clearance testing is required at the process characterization and process validation stages, and no specification testing is needed for lot release (Figure 18.1).

On the other hand, 3.7 mg/dose of MOPS is used as an elution buffer in the last column chromatography step of the purification process in this hypothetical process. The exposure limit based on ICH Q3A(R2) for MOPS is determined to be 1 mg/dose (Table 18.4), providing the estimated safety margin of 0.27. This result indicates that clearance testing is required to demonstrate that MOPS is cleared to a safe level. The process must remove MOPS at least 3.8-fold from the starting amount in order for this impurity to be below the safe level. Upon testing, 860 μg/dose of MOPS is observed in the final product, resulting in S_m of 1.2. While the amount of MOPS in the product is below its exposure limit, there is not sufficient latitude from the safety viewpoint. The small S_m value dictates that specification testing must be conducted for lot release (Figure 18.1).

The necessity of specification testing can be further verified by employing the FMEA risk assessment procedure. In this case, the process is only able to remove MOPS up to 4.4-fold, leading to the high occurrence score of 7 (Table 18.1). Multiplying the severity score of 9 (S_e), occurrence score of 7 (fold clearance), and detectability score of 7 (S_m) provides an overall FMEA score of 441. Such a high overall risk score confirms the requirement that specification testing for MOPS be included as part of lot release.

18.9 Summary and Conclusions

The purpose of this chapter is to demonstrate how ICH Q6B guidelines may be implemented in practice for reagent (process-related) impurities of biopharmaceuticals. A risk assessment involving a hypothetical process was described. The risk assessment begins with a toxicology assessment of the process reagents. This toxicology assessment is used to determine the residual limit of an impurity in the DS. The limit is calculated using the PDE (noncarcinogens or threshold-based carcinogens), AI (mutagens/mutagenic carcinogens), and the maximum daily dose. The methodology for deriving these limits is adapted from ICH Q3C(R2), ICH Q3D, and ICH M7. In some instances, a thorough toxicology analysis does not provide enough data to derive a limit. In these cases, the ICH Q3A(R2) limits are leveraged to derive a limit in the DS.

The requirement for specification testing for a given process-related impurity can be determined using a risk assessment (FMEA) approach. Such an assessment takes into account the amount of a process reagent added to the process, the process steps at which the process reagent is added and potentially removed, and the extent (fold) to which it is removed. These process data, in

turn, must be combined with the toxicology profile of the process reagent as a part of the risk assessment. The FMEA can provide an objective and defensible assessment of whether specification testing for a process-related impurity is required.

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References

1 International Conference on Harmonisation (ICH) 1999. Q6B. Specifications: Test procedures and acceptance criteria for biotechnological/biological products.
2 International Conference on Harmonisation (ICH) 2006. Q3A(R2). Impurities in new drug substances.
3 International Conference on Harmonisation (ICH) 2006. Q3B(R2). Impurities in new drug products.
4 International Conference on Harmonisation (ICH) 2011. Q3C(R5). Guideline for residual solvents.
5 International Conference on Harmonisation (ICH) 2014. Q3D. Guideline for elemental impurities.
6 International Conference on Harmonisation (ICH) 2014. M7. Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk.
7 Qiu J, Li K, Miller K, Raghani A 2015. Risk-based strategy to determine testing requirement for the removal of residual process reagents as process-related impurities in bioprocesses. *PDA J Pharm Sci Tech* 69:334–345.
8 Nema S, Brendel RJ 2011. Excipients and their role in approved injectable products: Current usage and future directions. *PDA J Pharm Sci Tech* 65:287–332.
9 Powell MF, Nguyen T, Baloian L 1998. Compendium of excipients for parenteral formulations. *PDA J Pharm Sci Tech.* 52:238–311.
10 Rowe RC, Sheskey PJ, Cook WG, Fenton ME 2012. *Handbook of Pharmaceutical Excipients*, 7th Edition, Pharmaceutical Press, London.
11 Howard L 2005. Enteral and parenteral nutrition therapy, In *Harrison's Principles of Internal Medicine* (Kasper D, Braunwald E, Hauser S, Longo D, Jameson L, et al. Ed.) 16th Edition, pp 415–422, McGraw-Hill, New York.

12 United States, Food and Drug Administration (US FDA) 2015. Inactive ingredient search for approved drug products. http://www.accessdata.fda.gov/scripts/cder/iig/getiigWEB.cfm (accessed April 7, 2017).
13 United States, Food and Drug Administration (US FDA) 2015. Food and Drugs: Substances Generally Recognized as Safe. Code of Federal Regulations, Title 21, Part 182. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=182 (accessed April 7, 2017).
14 Carcinogenic Potency Database 2015. The carcinogenic potency project. http://toxnet.nlm.nih.gov/cpdb/cpdb.html (accessed April 7, 2017).
15 Müller L, Mauthe RJ, Riley CM, Andino MM, Antonis DD, Beels C, DeGeorge J, De Knaep AG, Ellison D, Fagerland JA, Frank R, Fritschel B, Galloway S, Harpur E, Humfrey CD, Jacks AS, Jagota N, Mackinnon J, Mohan G, Ness DK, O'Donovan MR, Smith MD, Vudathala G, Yotti L. 2005. A rationale for determining, testing, and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity. *Regul Toxicol Pharmacol* 44:198–211.
16 Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F, Vos JG, Würtzen G 2004. Structure-based thresholds of toxicological concern (TTC): Guidance for application to substances present at low levels in the diet. *Food Chem Toxicol* 42:65–83.
17 International Conference on Harmonisation (ICH) 2015. M7(R1). Addendum to ICH M7: Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk, Current Step 2 version.
18 Dolan DG, Naumann BD, Sargent EV, Maier A, Dourson M 2005. Application of the threshold of toxicological concern concept to pharmaceutical manufacturing operations. *Regul Toxicol Pharmacol* 43:1–9.
19 Bercu JP, Sharnez R, Dolan DG 2013. Advancing toxicology in RiskMAPP: Setting ADEs based on the subsequent drug substance. *Regul Toxicol Pharmacol* 65:157–161.
20 International Conference on Harmonisation (ICH) 2009. Q8(R2). Pharmaceutical development.
21 International Conference on Harmonisation (ICH) 2012. Q11. Development and manufacture of drug substances (chemical entities and biotechnological/biological entities).
22 GE Healthcare, Medi-physics, Inc. 2013. Prescribing information for Indium In-111 oxyquinoline solution.
23 Pfizer Laboratories 2012. Prescribing information for methotrexate injection.
24 Williams HJ, Willkens RF, Samuelson CO Jr, Alarcón GS, Guttadauria M, Yarboro C, Polisson RP, Weiner SR, Luggen ME, Billingsley LM, Dahl SL, Egger MJ, Reading JC, Ward JR 1985. Comparison of low-dose oral pulse methotrexate and placebo in the treatment of rheumatoid arthritis. A controlled clinical trial. *Arthritis Rheum.* 28:721–730.

25 European Medicines Agency (EMA) 2014. Guideline on setting health based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities. EMA/CHMP/CVMP/SWP/169430/2012.

26 United States, Food and Drug Administration (US FDA) 2000. Summary basis of approval for Norditropin (Somatropin [rDNA origin] Injection). http://www.accessdata.fda.gov/drugsatfda_docs/nda/2000/21-148_Norditropin.cfm (accessed April 7, 2017).

19

ICH Q7 Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients (APIs)

Gordon Munro

Munro-Elbrook Associates, Welwyn, UK

19.1 Introduction and Principles on Which ICH Q7 Is Based

19.1.1 Introduction

In February 1998 the ICH Steering Committee decided to take up the proposed topic of producing a guideline on GMP for starting materials relating to active pharmaceutical ingredients (APIs) this being defined as phase 1. At the time of the inception of ICH Q7A [1], the intent was to have other phases that would create guidelines covering other topics including, excipients, source materials for biologicals, and target materials for radiopharmaceuticals. Later it was decided not to pursue these topics in favor of developing harmonized guidance for quality risk management (Q9) [2] and pharmaceutical quality systems (Q10) [3]. Regarding ICH Q7 an expert working group (EWG) was formed following the Steering Committee meeting in 1998 charged with creating the draft document relating to APIs. The membership included representatives of the then six members of ICH:

1) The US Food and Drug Administration (FDA)
2) The European Medicines Evaluation Agency (EMEA)
3) The Japanese Ministry of Health, Labour, and Welfare (MHLW)
4) The Pharmaceutical Research and Manufacturers of America (PhRMA)
5) The European Federation of Pharmaceutical Industries and Associations (EFPIA)
6) The Japan Pharmaceutical Manufacturers Association (JPMA)

The then normal ICH observers were Health Canada, the World Health Organization (WHO), the European Free Trade Association (EFTA), the

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International Generic Pharmaceutical Alliance (IGPA), and the World Self-Medication Industry (WSMI).

In addition, regulators from Australia, China, and India and the Pharmaceutical Inspection Cooperation Scheme (PICS) were also invited to attend meetings of the EWG. The composition of the membership demonstrated the increasingly global nature of the API industry and the wide range of interest around the world in the proposed guide.

The final draft was signed off by the EWG in November 2000 following extensive discussions and consultations. Implementation across the ICH regions and beyond followed over the next 18 months.

At the time, ICH Q7A was a landmark project in ICH because it was the first ICH guideline to address GMP. Once approved, it progressively directly replaced existing guidance such as that issued by PhRMA, WHO, FDA, PIC/PICS, and EFPIA/CEFIC (European Chemical Industry Council) with one harmonized document. Before Q7A, the interpretation of GMP for APIs was varied between regions and countries and among regulators and the regulated. Consequently among the prime aims of the guide were to produce a harmonized guide and equally importantly a shared, consistent understanding among regulators and the regulated industry. Both were intended to facilitate consistent compliance to support a uniformly high standard of assurance of the quality of APIs for incorporation into medicines supplied to human patients. This intention was further supported by a comprehensive training program created and delivered by members of the EWG at multiple venues around America, Europe, and Japan.

There was little that was new in ICH Q 7A, it effectively brought together much of the previously published guidance including that from FDA, PIC/PICS, and EFPIA/CEFIC. Using this approach ensured the consolidation and clarification of existing GMP principles and facilitated the implementation of Q7A. The most notable examples of new concepts are the section on agents, brokers, traders, distributors, repackers, and re-labellers, the formal introduction of the concept of impurity profiles, and the use of the term API starting material (as defined in Q7A) in determining where GMP, as set out in the guidance, should start to apply in the manufacturing process. Interestingly a risk-based approach was used in drafting the document before the creation of ICH Q9 [2]. This risk-based approach is evident in many areas of the text such as the definition and frequent use of the word critical in many sections.

19.1.2 Principles on Which ICH Q7A Is Based

The major principles on which ICH Q7A was written are:

1) While many of the GMP concepts, which apply to finished products, may apply to the manufacture of APIs, the application of such concepts might be different and the guidance should reflect this clearly. This reflects the difference between API and product manufacture. API manufacture combines

chemical and/or biological reactions that often allow purification with some physical processes, while manufacture of finished pharmaceuticals primarily involves physical processes where there is mainly no opportunity for further purification of the product. At the time that ICH Q7A was adopted, there was, perhaps surprisingly, no similar drive to develop a harmonized guide to GMP for finished pharmaceuticals. It is postulated that the reasons for this relate to the various national and regional codes of GMP, these being better developed and also enshrined in different legal systems making development of specific ICH guidance for products seem challenging and of marginal value.

2) The guidance should be a comprehensive document focused on GMP for the manufacture and distribution of APIs for human use, one which established a technically sound approach for applying GMP to such materials. It was not intended to cover veterinary products, registration, and filing requirements for APIs or pharmacopoeial requirements, vaccines, blood and plasma derivatives, gene therapy APIs, medicinal gases, bulk packaged medicinal products, or radiopharmaceuticals.
3) GMP as defined in the guidance should apply beginning with the point at which the API starting material (as defined in Q7A) starts to be used in synthetic manufacturing processes and on a case-by-case basis for others.
4) The manufacturer should define, justify, and document the point at which the production of the API begins unless this is defined in the relevant regulatory filing.
5) The level of control should increase the nearer the activity is to the isolation and purification stages of the API.
6) The use of the term critical, as defined in the glossary, to implement the concept of risk assessment/management into the guidance.
7) Critical process steps should be validated.
8) The word "should" when used in the text "identifies recommendations that, when followed, will ensure compliance with GMPs" in those countries/territories adopting Q7A as their standard although alternative approaches may be used as long as they meet regulatory and statutory requirements.

Before using ICH Q7A it is very useful to read the whole document and in particular the Glossary to obtain a clear understanding of the definitions of the key words/terms used throughout the guidance, for example, "critical," "API starting material," "quarantine," "reprocessing," and "reworking."

19.2 Key Features of ICH Q7A

19.2.1 Scope of Q7A

In understanding the scope of the guideline, a major key is table 1 in section 1. This shows the seven types of API manufacturing covered by the guideline and provides information on where the GMP, which Q7A defines, should

begin to be applied to each type. This must be read alongside the definition of the API starting material; also in section 1 of the guideline, this is referred to multiple times in the table.

The definition contains three major themes on what constitutes an API starting material:

1) "Used in the production of an API and that is incorporated as a significant structural fragment into the structure of the API"
2) May be "an article of commerce, a material purchased from one or more suppliers under contract or commercial agreement, or produced in-house"
3) "Normally have defined chemical properties and structure"

Although Q7A does not apply to stages of the manufacturing process before those highlighted in Table 19.1, this does not mean that there is no need to control those stages. It means that the manufacturer should define and apply standards of quality control/assurance appropriate to the materials and process(es) being used.

19.3 Quality Management

19.3.1 Principles

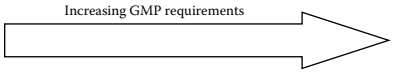
Much of the guidance in section 2 of Q7A as already stated was not new, but its articulation provided documentation of the expectations of staff and in particular the role of the quality unit for production activities. A decision was made not to use a term such as production or manufacturing unit to avoid being prescriptive about organizational requirements when there was no regulatory requirement to do so, and the tasks involved may be performed by a staff from a variety of departments, such as engineering, technical support or shared services, and production.

The key principles defined within quality management, apart from the obvious that quality should be the responsibility of everyone involved in manufacturing, that the quality unit is independent of production, and that quality is involved in all quality-related matters, are the following:

1) There should be a documented system for managing quality within which management and manufacturing personnel are actively engaged in addition to the quality unit. A concept developed more fully in the subsequent ICH Q10 Guidance: Pharmaceutical Quality Systems (03.)
2) The individuals authorized to release intermediates and APIs should be specified.
3) All quality activities are recorded contemporaneously, that is, at the time they are performed.

Table 19.1 Application of this guidance to API manufacturing (from ICH Q7A).

Type of manufacturing	Application of this guidance to steps (shown in gray) used in this type of manufacturing				
Chemical manufacturing	Production of the API starting material	Introduction of the API starting material into process	Production of intermediate(s)	Isolation and purification	Physical processing and packaging
API derived from animal sources	Collection of organ, fluid, or tissue	Cutting, mixing, and/or initial processing	Introduction of the API starting material into process	Isolation and purification	Physical processing and packaging
API extracted from plant sources	Collection of plant	Cutting and initial extraction(s)	Introduction of the API starting material into process	Isolation and purification	Physical processing and packaging
Herbal extracts used as API	Collection of plants	Cutting and initial extraction		Further extraction	Physical processing and packaging
API consisting of comminuted or powdered herbs	Collection of plants and/or cultivation and harvesting	Cutting/ comminuting			Physical processing and packaging
Biotechnology: fermentation/cell culture	Establishment of master cell bank and working cell bank	Maintenance of working cell bank	Cell culture and/or fermentation	Isolation and purification	Physical processing and packaging
"Classical" fermentation to produce an API	Establishment of cell bank	Maintenance of the cell bank	Introduction of the cells into fermentation	Isolation and purification	Physical processing and packaging



4) Any deviation should be documented and explained, critical deviations investigated, and conclusions documented.
5) Although normal practice should be to only release materials after completion of their evaluation by the quality unit, release under quarantine within a site or between operational units within the same organization is allowable provided there is a control system or systems in place to manage this including tracking of transportation and use.
6) A procedure to escalate to relevant management potentially significant quality events such as regulatory inspections, product defects, and serious GMP deficiencies.

19.3.2 Responsibilities of the Quality Unit

This section of Q7A provides a list of those activities that the quality unit should not delegate, using three concepts in particular. It is important to understand their meaning:

1) Release—Final authority lies with the quality unit to approve critical documents integral to the release process, for example, master batch production and laboratory records for APIs and the rejection or release of all APIs and intermediates to be used outside the manufacturing company.
2) Review and approval—Quality unit has accountability. But this may be shared with other individuals or groups provided any delegation of responsibility is clearly documented.
3) Accountability—As noted responsibility may be delegated but ultimate accountability remains with the quality unit. Where responsibilities are delegated this must be managed through appropriate systems and the quality unit should ensure the robustness of the system through self inspections. The quality unit must also oversee the investigation of any quality related complaints.

19.3.3 Responsibilities for Production Activities

Production activities may be conducted in a range of departments depending on the size, structure, and culture of the organization, so it is very important, as stated in Q7A, that the responsibilities for such activities "should be described in writing and should include, but not necessarily be limited to," the contents of the list provided:

1) "Preparing, reviewing, approving, and distributing the instructions for the production of intermediates or APIs according to written procedures
2) Producing APIs and, when appropriate, intermediates according to preapproved instructions

3) Reviewing all production batch records and ensuring that these are completed and signed
4) Making sure that all production deviations are reported and evaluated and that critical deviations are investigated and the conclusions recorded
5) Making sure that production facilities are clean and, when appropriate, disinfected
6) Making sure that the necessary calibrations are performed and records kept
7) Making sure that the premises and equipment are maintained and records kept
8) Making sure that validation protocols and reports are reviewed and approved
9) Evaluating proposed changes in product, process, or equipment including their approval before manufacturing is started
10) Making sure that new and, when appropriate, modified facilities and equipment are qualified"

Most activities on the list of responsibilities for production activities were commonly performed outside the quality unit before the publication of Q7A, and in using the list, it is important to remember that the concepts defined in Section 19.3.2 apply. This is demonstrated, for example, by item 1 on the list "Preparing, reviewing, approving, and distributing the instructions for the production of intermediates or APIs according to written procedures" where the use of "review and approval" indicates activities that are shared with the quality unit, for example, for master batch instructions. Another example is provided by the statement "Making sure that new and where appropriate, modified facilities and equipment are qualified." This is a clear example where a number of departments might be involved but those responsible for production activities would oversee and check that the tasks undertaken delivered the required outcomes.

19.3.4 Personnel

Section 3.1 of Q7A largely contains advice that still reflects current industry practice and common sense. However two areas that are often not given enough attention are:

1) Periodic review of training
2) The requirements for training consultants, which parallel those for permanent staff, including record keeping

Both points are pivotal to ensuring staff and consultants are properly trained and educated to perform their tasks in an efficient, effective manner while remaining in compliance with changing operational, quality, and regulatory requirements.

19.4 Buildings and Facilities

19.4.1 Buildings

Major aims of this section were to provide adequate guidance on key features for design and construction; utilities; containment; lighting, sewage, and refuse; and sanitation and maintenance, while reflecting the differences in these areas compared with those used for the manufacture of finished products.

Underlying themes behind the section are that buildings and facilities are often designed and built to allow manufacturing using different processes to protect operators and products and that the control of processes is more important when "open" systems are used.

The risk-based approach taken during drafting of Q7A is evident in the following:

- The provision that "where the equipment itself (e.g., closed or contained systems) provides adequate protection of the material, such equipment can be located outdoors."
- The emphasis on qualifying and monitoring utilities is confined to those that could impact quality such as those that come into contact with the product, for example, steam, gases, compressed air, and air delivered through HVAC systems including, in particular, where air is recirculated.
- The recommendation that unless otherwise justified process water should meet WHO guidelines for potable water [4] unless this is insufficient to ensure the quality of the API. An example of an application of this approach is the specific requirement for water used in the final isolation and purification steps to be monitored and controlled for total microbial counts, objectionable organisms, and endotoxins. In such instances the use of pharmacopoeial grade water, for example, USP purified water [5] or USP water for injection [6] (sterile API), is appropriate.

19.4.2 Containment

The requirements established in the containment section cover when dedicated production areas may be needed. In addition to the classical examples of highly sensitizing materials such as penicillins, they include:

- The need for procedures to be established and implemented to prevent cross-contamination from the movement of personnel and materials between areas.
- The manufacture of highly toxic non-pharmaceutical materials should not be in buildings and/or equipment used for APIs.

One of the more difficult challenges is in measuring and demonstrating the acceptability of precautions such as containment taken to prevent contamination.

Q7A does not provide specific advice on this topic. However the recent EMA guideline on setting health-based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities now provides comprehensive, useful advice in this area [7]. This is based on the principle of establishing a permissible daily exposure (PDE) limit for the residual compound using relevant preclinical data (e.g., a no observed adverse effect level (NOAEL)) and the equation for the derivation of the PDE presented in ICH Q3C [8].

19.4.3 Lighting, Sewage, and Refuse

The final short paragraphs in section 4 of ICH Q7 covering lighting, sewage, and refuse and sanitation provide general statements of fundamental requirements to ensure that manufacturing areas and equipment are kept properly maintained and cleaned.

Although the requirements may seem obvious, they are nevertheless often neglected with adverse consequences to product quality, for example, when rodenticides, insecticides, and sanitizing agents are used, written procedures should be created and followed.

19.5 Process Equipment

19.5.1 Design and Construction

The short initial paragraph in section 5.1 of ICH Q7 is an excellent though high-level summary of the critical quality requirements defined in more detail in the subsequent paragraphs. While all are important, three often neglected in implementing Q7 are that: "Any substances associated with the operation of equipment, such as lubricants, heating fluids or coolants, should not contact intermediates or APIs so as to alter the quality of APIs or intermediates beyond the official or other established specifications. Any deviations from this practice should be evaluated to ensure that there are no detrimental effects on the material's fitness for use. Wherever possible, food grade lubricants and oils should be used." A key point in this paragraph that is often not fully understood or is misinterpreted is that while it is not desirable for substances associated with the operation of equipment to come into contact with APIs or intermediates, in practice the presence of low levels of such materials is very difficult if not impossible to avoid 100% of the time. Hence, the important caveat is that any such contact should not "alter the quality of APIs or intermediates beyond the official or other established specifications." The last sentence in the Q7A paragraph quoted here provides specific advice on a means of simplifying any investigation into the impact of low residual levels of substances associated with the operation of equipment. In addition,

- "A set of current drawings should be maintained for equipment and critical installations, e.g. instrumentation and utility systems and
- Closed or contained equipment should be used whenever appropriate. Where open equipment is used, or equipment is opened, appropriate precautions should be taken to minimize the risk of contamination."

19.5.2 Equipment Maintenance and Cleaning

The main theme relating to this topic—The need for written preventative maintenance schedules and procedures and cleaning procedures to be in place and in use—is common to both the manufacture of APIs and formulated pharmaceuticals. There are, however, two very important differences underlying the approach to cleaning taken in Q7A reflecting the difference between the manufacture of API and formulated pharmaceuticals. These two are that many if not most of the early stages in API manufacture include chemical or biological processes and the final steps are often dedicated to purification. This is in contrast to formulated pharmaceuticals where most operations are physical manipulations that do not present opportunities for purification. Consequently cleaning during the manufacture of APIs may be directed at processing steps identified as those with the greater risks of cross-contamination or carry-over of impurities into the quality of the finished API.

Among the requirements within this section, some of the ones most often neglected are the following:

- The need to include the dilution requirement for cleaning agents in procedures
- Complete instructions for the disassembling and reassembling of equipment during the cleaning processes
- The need for cleaning of equipment used continuously on a campaign basis at appropriate (defined and justified) intervals to prevent build up and carry-over of contaminants such as degradants and the need to set limits for residues of cleaning agents [7] (Requirements for the validation of cleaning procedures are dealt with in section 12.1 of Q7A.)

19.5.3 Equipment Calibration

The fundamentals of calibrating control, weighing, measuring, monitoring, and testing equipment in Q7A are generally well understood and followed. The need for and the conduct of investigations into deviations from the approved calibrated state is less well understood, in particular the evaluation of the impact of such deviations on all lots of intermediates and/or APIs manufactured using the equipment since its last successful calibration. Evaluation of the risk should be based on thoroughly assessing the quality/compliance implications and not be simply focused on justifying forward processing or release of material.

19.5.4 Computerized Systems

This section provides a very high-level statement of GMP requirements but little detail of how to meet them in practice. The ISPE GAMP Guide [9] is now widely recognized as providing the most informative best guidance on this subject.

19.6 Documentation and Records

This section provides the core overall definition of the requirements for the documentation system and specifications for production and laboratory control records related to batch manufacturing. This is supplemented by information in the subsequent sections dealing with the activities necessary to support successful manufacturing and identification labeling of APIs and intermediates, validation, etc. Important considerations in successfully implementing these requirements include the following:

- Having a clear definition of the length of time for which documents have to be retained (generally where the expiration dating of the finished pharmaceuticals derived from the API is known, the retention period is 1 year longer than the expiration of the last batch of finished product derived from the specific batch of API). However many organizations keep documents for much longer, for example, until the product is withdrawn from all markets and all stock is depleted and beyond the expiration date of the last lot released to the market. This is often done to support possible future litigation.
- Where records are retained in a medium other than the originals, they must be true verified copies and the means of retrieving them must be maintained throughout the retention period of the documents. When organizations retain records for longer than the minimum requirements, it is particularly important to ensure that records are kept on/in a stable medium because paper copies may fade. In addition if a non-electronic means of storing documents is selected, consideration has to be given to providing suitable, adequate capacity for long-term storage of the large volume of documents.
- Ensuring the guidance on the responsibilities of the quality unit and those for production activities defined in section 2.2 are applied to the creation, control, and use of documentation.
- There is no single preferred or correct system for master and production batch records (or any others) as long as the criteria set out in the relevant sections of Q7A are met.

Some other neglected or misunderstood points are that:

- Yields should be set as ranges but may vary, for example, between the first and last batches of a campaign where “heels” are being laid down or harvested.

- Instructions for the storage of intermediate or APIs including labeling, packaging, and storage conditions where time limits need to be established, documented, and followed.
- Batch records and laboratory control records for critical process steps should be reviewed and approved by the quality unit before an API batch is released or distributed. (The quality unit can however delegate the responsibility and authority for release of intermediates unless they are to be shipped outside the control of the manufacturing company.)
- All deviations must be noted (recorded) and critical deviations investigated including all other batches that might be impacted.

19.7 Materials Management

To understand the scope and how to interpret this section, it is advisable to consult the Glossary and review the relevant definitions (reproduced here to help the reader), in particular "critical," "API starting material," "material," "raw material," "process aid," and "quarantine."

Critical

Describes a process step, process condition, test requirement, or other relevant parameters or items that must be controlled within predetermined criteria to ensure that the API meets its specification.

API Starting Material

A raw material, intermediate, or an API that is used in the production of an API and that is incorporated as a significant structural fragment into the structure of the API. An API starting material can be an article of commerce, a material purchased from one of more suppliers under contract or commercial agreement or produced in-house.

Material

A general term used to denote raw materials (starting materials, reagent, and solvents), process aids, intermediates, APIs, and packaging and labeling materials.

Raw Material

A general term used to denote starting materials, reagents, and solvents intended for use in the production of intermediates or APIs.

Process Aids

Materials, excluding solvents, used as an aid in the manufacture of an intermediate or an API that do not themselves participate in a chemical or biological reaction (e.g., filter aid, activated carbon, etc).

Quarantine

The status of materials isolated physically or by other means pending a decision on their subsequent approval or rejection.

Using these definitions it is clear that while there needs to be a specification for all materials (starting materials, intermediates, reagents, solvents, process aids, APIs, and packaging and labeling materials), ICH Q7A makes mandatory the evaluation of suppliers of critical materials, that is, any material that must be "controlled within predetermined criteria" to ensure that the API meets its specification.

Other key features of materials management are that all supplies of "materials" should be purchased from a supplier or suppliers approved by quality; each container or group of containers must be visually examined on receipt for accuracy of labeling and damage and held under quarantine until sampled/examined/tested. Importantly ICH Q7A allows that quarantine may be physical or by other effective means such as a secure computer-based materials management/warehousing system. However some regulators can be difficult to convince that physical segregation is not required due to local regulations or their interpretation.

Bulk deliveries from non-dedicated tankers merit particular attention to avoid the risk of cross-contamination. Means of achieving the required levels of control include certificates of cleaning, testing for trace impurities, and audit of suppliers and the transport company providing the tankers.

An identity test should be performed on each batch of material with certain exceptions such as processing aids, hazardous or highly toxic materials, and materials transferred from another unit in the same company. Any such exceptions should be supported by a documented justification (e.g., for a processing aid a case may be made based on the established inert nature of the material, and for highly reactive materials user tests may be appropriate.) The results of the ID test with a supplier C of A can be used to release the material.

Storage of materials outdoors is subject to the use of suitable containers and their being legibly labeled. The containers should be cleaned before the material they contain is used.

The minimum requirement for supplier approval in Q7A is that adequate evidence including full analyses on at least three batches is completed satisfactorily before reducing the level of testing with full analyses versus the C of A due at predetermined intervals. During the time since Q7A was approved and adopted, supply chain integrity and supplier quality management have attracted

more attention, and requirements have been defined more fully (e.g., see References [10–12]). In particular quality agreements or quality sections in technical agreements setting out the responsibilities of both contract donor and acceptor are defined. In this context change control, right of access to conduct audits, and final approval of APIs for release are pivotal.

Sampling, testing, and storage requirements largely follow those long in place in finished product manufacturing. Two areas that need particular attention are the requirement to sample at defined locations to prevent contamination and the specific clause on reevaluation, indicating that materials be reevaluated as appropriate to determine their suitability for use, for example, after prolonged storage or exposure to heat or humidity. The "after prolonged storage" example allows the reevaluation and creation of a documented justification for using materials that otherwise might be discarded. However it is important that reevaluation does not become routine to compensate for poor practices in obtaining and maintaining the supply of materials.

19.8 Production and In-Process Controls

The focus of this section is on production operations, final limits, in-process sampling and controls, blending of batches of intermediates and APIs, and contamination control. Some of the key factors in successfully following the Q7A guidance are as follows:

- Critical weighing, measuring, or subdividing operations are witnessed (interpreted as supervisory but this could be by peers) or subject to an equivalent control—equivalent control means confirmation by a second independent means, for example, printout from an electronic or mechanical source. A prime purpose of such double checks is to ensure that the integrity of records is maintained, particularly when changes are made.
- Actual yields should be compared with expected yields based on historic performance data and any deviation documented and explained; deviations at critical steps require documented investigation that assesses their impact on the quality of the affected batches.
- Time limits for completion of individual processing steps and/or the total process should be established in the master batch record, and deviations from the limits should be documented and evaluated. Such limits may be inappropriate during processing to a target value such as drying to a predetermined specification where control is exercised through a specified limit, for example, the residual solvent/water level.
- In accord with the guiding principle embedded in ICH Q7A, in-process testing may be less stringent in early processing with tighter controls later in the process. All in-process tests should reflect the nature of the API being manufactured and the impact that the particular step/stage in the process has on the API. This aligns to concepts outlined within ICH Q11 [13] in

terms of API critical quality attributes and the properties of materials that influence them.

- Blending of intermediates and APIs is an important process and is defined as the process of combining materials within the same specification to produce a homogeneous intermediate or API. This means that materials that do not meet specification cannot be blended. The in-process combining of fractions from several batches for further processing or the mixing of fractions from a single batch is not considered as blending. Continuous manufacturing poses additional challenges in assessing whether material produced consistently meets specification. These are best addressed during validation by increased monitoring, leading to a defined and documented justification for in-process sampling plans. Finished product testing may be addressed by defining batches by elapsed manufacturing time.

  All batches introduced into a blend should be made using the established process and the blend tested for compliance with specification. APIs where physical properties are critical to the subsequent use of the blending process should be validated to determine homogeneity of the batch.

  Stability testing should be performed if the process of blending might affect stability and the expiry or retest date of the blended batch should be based on the date of manufacture of the oldest input material.
- Residual materials can be carried over from one batch to another of the same intermediate or API subject to adequate control. Any such carry-over should be demonstrated not to adversely alter the API or intermediate impurity profile. This is particularly relevant to justifying not performing cleaning between batches during campaign working. Production operations should all be conducted in a way that prevents contamination with particular emphasis on stages after the final purification steps of the API.

19.9 Packaging and Identification Labeling of APIs and Intermediates

The fundamental principles embedded in this section are the need for containers to be fit for their purpose and provide adequate protection against deterioration or damage, that labels are tightly controlled both during storage and use, and that labeling provides clear identification and traceability. Traceability is of particular importance when material is shipped outside of the control of the quality system of the manufacturing company in which it originated and there are specific requirements defined concerning what is expected.

Some of the specific requirements that are more commonly neglected are that packaging materials should not react with the materials that they are used to store, nor should they absorb or add anything, for example, by leaching. While contamination through leaching is unlikely when the stored material is a dried solid, an assessment should still be conducted. If containers are reused,

they must be thoroughly cleaned according to documented procedures and all previous labels must be replaced or defaced.

Control of labels and printed packaging materials is often deficient, with neglect over control of access to storage areas, excess labels with batch numbers and obsolete labels not destroyed in a timely fashion, and printing devices used to print labels for packaging operations not controlled adequately to make sure that the print complies with that specified for the batch for which they are used.

19.10 Storage and Distribution

Apart from the general expectation that facilities for storage of all materials, including temperature and humidity control, are adequate and that records are maintained, there are a number of other points that are worth noting:

- Any rejected material, returned goods, and recalled material should be held in quarantine.
- Defined quarantine areas should exist unless there is a system that provides equivalent security, for example, secure computerized random location.
- Materials may be shipped under quarantine from site to site or within a site controlled by the same company following approval for the transportation by the quality unit. Supply to a third party under quarantine is not allowed. Both intermediates and APIs should be released by the quality unit.
- It is the duty of the manufacturer to ensure that the provider of the transport used to ship APIs and intermediates outside the company's control complies with all relevant transport and storage conditions. These should be defined during product development and be part of the technology transfer package if manufacture is transferred between sites including those of contract manufacturers. For companies seeking to sell their products in Europe, it is essential that they check the impact of and when necessary ensure that they comply with the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulations [14]. This involves creating an inventory of every chemical that comes into, is part of, and goes out of the business, assessing their hazardous properties, record keeping, and registration requirements.
- There should be a robust system in place to permit a recall if needed including full traceability to all customers and escalation routes within the company and to regulators.

19.11 Laboratory Controls

There are no fundamental differences between the requirements and expectations for good practice in testing laboratories for API manufacture and those supporting the manufacture of pharmaceutical dosage forms.

Despite the basic similarities in practices, one area where there is greater emphasis in API GMP is impurities and impurity profiles. This is because there are generally more potential residues, contaminants, and by-products arising from API manufacturing than in dosage form production. Due to their nature, many process related impurities are highly, for example, if mutagenic reagents are used in or generated during the manufacturing process they require control to very low levels. This is examined in Chapter 24 of this book relating to ICH M7 [15]. For reasons such as these, the section on impurities and impurity profiles is worthy of particular attention. In summary ICHQ7A states that an impurity profile should be established for every API (not normally necessary for APIs from herbal or animal tissue sources), this includes the specific identity, when known, or some other means of facilitating comparisons from batch to batch, such as retention or relative retention time, the normal range observed. The type of impurity, such as solvent, by-product, degradant, or inorganic impurity, should also be recorded. Useful additional, more detailed guidance on impurities is contained in ICH Q3A [16], Q6A [17], Q6B [18], and Q1A (R2) [19]. The impurity profile is usually specific to a controlled method of synthesis and should be generated from one of the more typical batches.

ICH Q7A recommends that the impurity profile of batches should be compared at appropriate intervals with the impurity profile in the regulatory filing or historical data. However it is good practice to do this as part of approving each batch for release. This is increasingly recommended/requested during regulatory inspections.

All analytical methods should be validated to ICH Q2A and Q2B (now ICH Q2R1 [20]) standards, the degree reflecting their purpose, meaning that the level of validation should be increased with the nature of the materials being tested, and where they are used in the process with greater impact, for example, on a critical attribute of an API or critical intermediate.

There is an exception to the requirement to validate when pharmacopoeial methods are used, but these, like all analytical tests, should be demonstrated to work in practice on the material being tested.

A requirement sometimes overlooked is the need to maintain records of all modifications to validated analytical methods including the data to verify that the results obtained using the modified method are as reproducibly accurate as the method before it was amended.

There is a section on stability monitoring of APIs, and it is important to note that this is a program aimed at monitoring ongoing routine manufacturing and should not be confused with stability testing to generate data to support process changes.

The purpose of the program is to monitor the stability characteristics of the APIs and to confirm their designated storage conditions and retest or expiry dates. This program also provides an additional means for detecting "drift" in processes or materials that impact product quality.

To have any relevance to the stability of the bulk API, it is very important that the container used to store the samples for testing mimics those used for full-scale manufacture, for example, the same material should be used for bags and containers on the small scale as are used on the manufacturing scale.

Normally the first three production batches are included in the predefined and documented program and at least one further lot per year unless, of course, none is produced in any given year. APIs with short shelf lives may require more frequent testing than the minimal annual requirement, which is applied to more stable APIs.

There is no requirement for accelerated storage studies in ICH Q7A although this is required for the overall evaluation of new drug substances for registration applications as set out in ICH Q1A [19].

In addition to the stability monitoring program, there is a separate requirement to hold reserve samples for future evaluation of the quality of each batch of API manufactured. As with the stability monitoring samples, these should be held in packaging that is the same as that used for the bulk API or one that provides at least as much or more protection. Sufficient material must be held to allow a minimum of two full analyses to specification. Samples should be maintained for 3 years after the expiry date as specified by the manufacturer or 3 years after it has been completely distributed, whichever is longer. Other factors may however dictate longer retention periods, but these should reasonably reflect stability.

Where possible it is advisable to keep reserve samples for 1 year beyond the shelf life of the last batch of finished dosage form in which the API was incorporated, but this may be difficult where the shelf life of all forms is not known.

ICH Q7A allows the use of both retest and expiry dates and provides guidance on how these should be derived and supported. In particular when an intermediate is to be shipped outside the manufacturing company's control, an expiry or retest date must be assigned and included on the labeling and in the certificate of analysis.

19.12 Validation

Although there has been continuing discussion concerning validation since ICH Q7A was completed, most of the guidance it provided is still highly relevant as it represents a pragmatic, predominantly science-based approach. Most if not all of the approaches, definitions, and documentation requirements should be readily recognized by anyone with experience of the manufacture of medical products. Examples of this are the requirement for validation protocols and reports, DQ, IQ, OQ, and PQ and the three permitted approaches to process validation—prospective, concurrent, and retrospective. The fundamentals of all

three remain relevant today although the need for retrospective validation, 13 years after the adoption of ICH Q7A that states that prospective validation should normally be performed, is questionable.

The guidance also deviated somewhat from the norm of the time of using three as the number of batches needed for prospective or concurrent validation by calling attention to the need to consider additional factors such as the complexity of the process or change being made, to decide the most appropriate number. It also includes a requirement for periodic review of systems and processes to confirm that they are producing material meeting specifications and established impurity profiles.

ICH Q7A reflects the differences between the manufacture of medicinal products and API. For medicinal products all manufacturing steps need to be validated, whereas for APIs it is only critical processing steps that have been assessed as impacting the quality and purity of the API. This aspect is examined in detail in ICH Q11 [13], in particular section 5—Selection of Starting Materials. It should be recognized that this can be a challenging area, one explored within Chapter 23 of this book relating to ICH Q11 [13].

Key features in the section on cleaning validation are that it should be focused on situations or process steps where contamination or carry-over of materials poses risk to the quality and purity of the API or intermediate. Where an intermediate or API is selected as representative of cleaning validation for a range of such materials, the selection should be based on solubility and difficulty of cleaning; and sampling may be by swabbing, rinsing, or an alternative method, in particular, to detect insoluble residues. Useful information on this is provided in the EMA shared facilities guideline [7].

It should also be remembered that where there is a need to control the bioburden for non-sterile APIs to be used in the manufacture of sterile APIs, then cleaning validation should include microbiological and if necessary endotoxin testing.

19.13 Change Control

The significance of change control is generally well understood; however it is also a common source of problems when poorly planned or executed. The guidance in the Q7A guidance is short but very focused including a requirement to have a formal change control system for all changes that could affect the production and control of the intermediate or API. All GMP relevant changes should not only be drafted, reviewed, and approved by the appropriate organizational unit but also reviewed and approved by the quality unit. It is critically important that changes at contract manufacturers are subject to a change control system and reported to the contract donor for final assessment/approval.

Three requirements that are sometimes not performed to acceptable standards are:

- Documentation, particularly the justification for categorization of changes.
- Not all documents impacted by the change are reviewed in a timely manner.
- The potential for actual changes to impact expiry or retest dates is not thoroughly evaluated including failing to conduct accelerated stability trials when needed.

However another practice that may arise from poor assessments of the critical risk from changes is placing too many samples on stability. Companies rather than conduct a thorough risk evaluation place samples on stability as a short-term means of saving time. Since an inadequate risk assessment was done, there is the chance that stability testing may not be an appropriate means of assessing the change or that no or fewer stability samples would have been acceptable.

Finally in the section, all critical changes whether planned or unplanned should be notified to all the manufacturers of medical products into which they have been incorporated so that they can assess any potential impact on their operations/products.

19.14 Rejection and Reuse of Materials

A fundamental principle in this section is that any intermediate or API that does not meet the requirements of its specification should be quarantined.

There are two options following rejection:

1) *Reprocessing*: putting an intermediate or API back into the process and repeating a step or steps that are part of the established process. If reprocessing becomes a common occurrence, it should be incorporated in the standard manufacturing process.
2) *Reworking*: taking an intermediate or API and performing one or more processing steps which are not part of the established process but which are filed with the relevant regulatory authorities for the markets to which they are supplied.

Recovery of reactants, intermediates, API, and solvents is allowed, provided that approved procedures exist (and are filed) and that the recovered materials meet appropriate specifications. ICH Q7A allows the use of recovered solvents in the same or different processes, provided that the process is properly controlled and monitored. In addition mixing recovered solvents with fresh supplies is allowed, provided that the use of the recovered material has been proved to be satisfactory. Establishing such proof can be very difficult if not

impossible particularly for highly toxic or sensitizing materials such as cytotoxics or penicillins. Consequently, in practice, material recovered from such processes is often only used in the process from which it was recovered.

One source of material for reprocessing and reworking is intermediates and APIs returned to the manufacturer. This is permissible subject to containers being in a satisfactory condition, such as seals and labels intact and data being available to show that the material has been stored and shipped correctly. Whatever the decision made on returned goods, reprocess, rework, or destroy, records need to be created and maintained, setting out the decision with sound justification.

The use of all recovered materials must be accurately documented.

19.15 Complaints and Recalls

This section is simple, precise, and easily followed. One requirement sometimes neglected is that not only should records of complaints be kept but also trends should be monitored and evaluated to ensure that actions can be taken when necessary to mitigate negative trends either to correct an out-of-compliance situation or better to prevent any arising. Despite the relative simplicity of the requirements, the number of negative findings in both regulatory and other audits suggests that many organizations do not pay sufficient attention to establishing and maintaining their complaint and recall systems. This may be due to the relatively low level of complaints received by API manufacturers.

19.16 Contract Manufacturers (Including Laboratories)

Contract manufacturers are defined in ICH Q7A as "a manufacturer who performs some aspect of manufacturing on behalf of the original manufacturer." This section should apply to all manufacturers covered by the definition with special emphasis on preventing cross-contamination and maintaining traceability—two areas that remain as important today as when the guidance was written.

The text sets out high-level, key statements that, if followed, provide a robust framework for well-controlled, documented, agreed ways of working between the contract giver and its receiver. Given the volume of API manufacture now done outside of the ICH area, it is critically important that companies using contract manufacturers know and understand the requirements and ensure they are understood and followed by their contract acceptors. One specific area that is critically important yet often not covered adequately is change control.

19.17 Agents, Brokers, Traders, Distributors, Repackers, and Relabeling

This was the only completely new section in the Q7A guidance compared with other regulations and guidelines and reflected concerns that arose in particular from regulators around the world, including the WHO. The title reflects the various nomenclatures known at the time to describe the organizations to which the section applies, but it is not a comprehensive list. The definition in the text "any party other than the original manufacturer who may trade and/or take possession, repack, relabel, manipulate, distribute, or store an API or intermediate" is intended to ensure that whatever name is used, the activities listed are subject to GMP as described in ICH Q7A.

There are a number of key requirements that should be given particular attention:

- Prevention of cross-contamination
- The responsibility of sharing information—in both directions
- Preventing the deterioration of products during all activities being conducted
- Maintaining traceability

All four points were emphasized because they were known to be areas where issues had previously arisen.

Other requirements often not conducted well and that should be addressed are keeping and making available to customers the name and address of the original manufacturer, keeping authentic certificates of analysis, maintaining records of all complaints and reviewing them when necessary with the original manufacturer, and ensuring that if an API or intermediate is repackaged differently from the original manufacture, adequate stability data are available to support the allocated expiry date in the new pack.

19.18 Cell Culture/Fermentation (CCF)

Key steps in the successful use of this section are an understanding that:

- It is not a stand-alone section but should be viewed as addressing additional issues and guidance for CCF. In addition ICH guidelines Q5A (Chapter 10 within this book) [21] and Q5D (Chapter 13) [22] should be read in conjunction with Q7A particularly where viral safety concerns and/or cell banking is involved.
- The guidance does not apply to the establishment of master and working cell banks, nor maintenance of the cell bank for classical fermentation although other GMP requirements may apply.

- The overall Q7A guidance does not apply to vaccines, whole cells, whole blood and plasma, blood and plasma derivatives, and gene therapy APIs but does include APIs that are produced using blood or plasma as raw material.

In the context of the previous three points, the first general section provides an introduction and overview of process controls. This is followed by four sections (cell bank maintenance and record keeping; cell cultures/fermentation; harvesting, isolation, and purification; and viral removal/inactivation steps) that provide more detailed guidance on the specific additional/different requirements for CCF for APIs.

The differences in the guidance reflect the nature of APIs manufactured using CCF that the production methods employed require more control, that there is a risk of contamination with potentially infectious agents, and that change in manufacturing process may result in different unexpected consequences for product quality than in the manufacture of APIs by chemical synthesis.

19.19 APIs for Use in Clinical Trials

This section recognizes the fact that the controls defined in the earlier sections of ICH Q7A are not all applicable to the manufacture of APIs for use in clinical trials when processes and controls are still being developed. Nevertheless GMP concepts should be applied with controls reflecting the stage of development of processes, analytical methods, and the available knowledge of the intermediates and APIs. Basic standards of control should be in place such as equipment used is calibrated, clean, and fit for its intended purpose; precautions should be taken to minimize the risk of contamination and cross-contamination. Documentation is still necessary but may be in laboratory notebooks, batch records, or other similar approaches. Yields may be more variable, and validation in the early stages of development may be replaced by a combination of controls, validations, and equipment qualifications. However, whenever batches are produced for commercial use, process validation should be performed irrespective of the scale of the plant/equipment used.

While change is to be expected during development, it should be documented and maintained as part of the history of the process/test/method/intermediate/API. Analytical methods need not be validated but should be scientifically sound.

While quality functions such as testing may be done in other organizational units such as R&D, there is still a requirement for an independent quality unit to release batches.

Overall levels of control and applications of full ICH Q7A GMP are expected to increase with process knowledge, understanding, and the transfer from pilot to large-scale manufacturing.

19.20 ICH Q7A Q&As

In October 2012 the ICH Steering Committee approved the establishment of an implementation working group (IWG) to address perceived uncertainties relating to the interpretation of some sections of ICH Q7A. Specific issues for clarification were identified:

- The application in the supply chain, control, and contractor/supplier management (outsourcing)
- Monitoring of impurity profiles
- Quality systems
- Applicability to biologicals/biotech and relationship with ICH Q5D
- GMP expectations in the development phase (manufacturing for clinical trials)
- The impact of the implementation of ICH Q8/11, Q9, and Q10 on Q7

A major input to the work of the IWG was to be the experience of the PIC/S in its implementation of ICH Q7A as documented in the Q&As created by the PIC/S Expert Circle on APIs.

Reading the ICH Q&A document approved by the ICH Steering Committee in June 2015 [23], it is difficult to assess how many of the specific issues were adequately addressed. For example, there is only one specific Q&A (11.1) that deals with impurity profiles; change control has only one specific section (13.1); GMP expectations in the development phase are only mentioned indirectly in paragraph 19.1 in a Q&A relating to the use of manufacturing equipment.

Overall the Q&As may add value in helping to standardize the interpretation of Q7A by regulators and industry. Unfortunately given the changes that have occurred since the approval of ICH Q7A in 2000, such as greater emphasis on supplier quality, changed approaches to validation, the use of risk-based approaches within GMP systems it may feel that the opportunity to enhance the value of the Guidance by updating the sections described, may have been missed.

19.21 Conclusions

Fourteen years after its approval, ICH Q7A remains highly relevant and is widely accepted globally by both the pharmaceutical industry and its regulators. Although it predates ICH Q8 [24], Q9 [2], Q10 [3], and Q11 [13], the

document is an excellent fit with their intent and content while providing information specific to APIs that the others do not.

ICH Q8 [24] and Q11 [13] provide very useful information on the technical and scientific information, which is the foundation for manufacturing consistently acceptable APIs and finished product. Q9 provides guidance on risk-based approaches that are applicable throughout a product life cycle including API manufacture, and Q10 [3] provides a sound approach to development and maintaining an overarching quality system that covers the product life cycle from development to discontinuation. In doing so ICH Q10 encompasses all the facets of GXP, of which GMP for APIs (Q7A) is an integral and very important part.

The recent approval of ICH Q7 Q&As may further help to maintain consistent interpretation of the original document although in some ways it may be viewed as a missed opportunity to provide more information in key areas that have assumed added importance over the lifetime of Q7, for example, supplier quality, supply chain integrity, and application of risk-based approaches to decision making related to quality systems or intermediates and APIs.

Given the continuing success of ICH Q7, it may be time to revisit the decision in 2003 not to pursue any other GMP-related topics through ICH. Although there is published guidance on topics such as excipients, supplier quality, and contract manufacture, the benefits of standards agreed by the world's major regulatory and industry representatives as demonstrated by Q7 should not be overlooked.

References

1 ICH Q7A—Good Manufacturing Practice for Active Pharmaceutical Ingredients, November 2000.
2 ICH Q9—Quality Risk Management, November 2005.
3 ICH Q10—Pharmaceutical Quality System, June 2008.
4 WHO Guidelines for Drinking Water Quality 4th Edition, 2011.
5 Purified Water, USP 39/NF34, November 2015.
6 Water for Injection USP39/NF34, November 2015.
7 Guideline on setting health based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities, European Medicines Agency 2015.
8 ICH Q3C (R5) Impurities: Guideline For Residual Solvents, February 2011.
9 GAMP5 A Risk Based Approach to Compliant G&P Computerised Systems Guide ISPE 2008.
10 FDA Guidance for Industry—Contract Manufacturing Agreements, draft guidance, May 2013 (final version—issued November 2016).
11 European Commission, GMP for Medicinal Products for Human and Veterinary Use, Chapter 7, Outsourced Activities, June 2012.

12 The International Pharmaceutical Excipients Council Quality Agreement Guide and Template 2009.
13 ICH Q11—Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/Biological Entities May 2012).
14 Regulation(EE) No 1907/2006 of the European Parliament and the Council of 18 December 2006, concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) establishing a European Chemicals Agency.
15 ICH M7—Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to limit Potential Carcinogenic Risk, June 2014.
16 ICH Q3A(R-2)—Impurities in New Drug Substances, October 2006.
17 ICH Q6A Specifications—Test Procedures and Acceptance Criteria for New Drug Substances and New Products—Chemical Substances, October 1996.
18 ICH Q6B Specifications—Test Procedures and Acceptance Criteria for Biotechnological/Biological Products, March 1999.
19 ICH Q1A(R-2) Stability Testing of New Drug Substances and Products Q1A(R2), February 2003.
20 ICH Q2R1 (previously coded Q2A, Q2B)—Validation of Analytical Procedures: Text and Methodology, October 1994/November 1996.
21 ICH Q5A R1—Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human and Animal Origin, September 1999.
22 ICH Q5D—Derivation and characterisation of substances Used for Production of Biotechnologicals/Biological Products, July 1997.
23 ICH Q7 Q&As Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients, Questions and Answers, June 2015.
24 ICH Q8 (R2)—Pharmaceutical Development, August 2009.

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Pharmaceutical Development

Per Holm, Morten Allesø, Mette C. Bryder, and René Holm

Chemical and Pharmaceutical Research, H. Lundbeck A/S, Valby, Denmark

20.1 Introduction

Pharmaceutical products are designed to be high-quality products developed to consistently relieve or cure diseases in patients with a defined need. According to good manufacturing practice (GMP), such products are required to be fit for their intended purpose, comply with the requirements of the marketing authorization (MA) or clinical trial authorization (CTA), as appropriate, and not place patients at risk due to inadequate safety, quality, or efficacy [1]. Historically, this compliance has been ensured using quality control (QC) approaches. However, benchmarking of the pharmaceutical industry with other industries has highlighted that the existing approach has a number of unwanted side effects, such as innovation inertia, an increased documentation burden for both the pharmaceutical industry and the regulatory agencies. This in turn led to the introduction of Quality by Design (QbD) [2, 3]. The basic rationale behind the QbD concept was that most quality problems relate to the initial product development, and there is a resultant reluctance with regard to continuous improvements for existing marketed products. Hence, quality should be designed into the product [4, 5], and a more flexible system needed to be established for post-approval changes. Therefore at the beginning of the 21st century, regulators started to encourage the pharmaceutical industry to gain better understanding of how the formulation and manufacturing process interrelates and impacts on product quality. Recent regulatory guidelines for the pharmaceutical industry, including FDA's Critical Path Initiative [6] and the process analytical technology (PAT) initiative [7] together with the promotion of QbD in the CMC pilot program [8, 9], have provided a broad strategic input on how the pharmaceutical industry can improve their production

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process and thereby the resultant products [10]. In particular, the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has provided a generalized direction on the path toward a pharmaceutical product, including the quality systems needed to support it via the establishment of the interrelated guidelines ICH Q8(R2) [11], Q9 [12], Q10 [13], and Q11 [14].

ICH Q8(R2) [11] introduces a number of new regulatory concepts into pharmaceutical development activities, and design space and design of experiments (DoE) have gained significant prominence in the scientific literature [15–17]. However, what has received significantly less focus is the use of prior knowledge, defined as documented knowledge within the company, and the potential use of technology platforms. Consequently, less guidance is available for industry in this important area. The aim of the current chapter is to explain the concept of ICH Q8(R2) [11] and QbD, in particular, on how to conduct a rational pharmaceutical development process with increased effectiveness and efficiency during both development and process validation phases within the framework defined by the ICH Q8(2) [11] and ICH Q9 [12] guidelines. This will be achieved using the concept of prior knowledge and the practical application of first-principles models to enhance mechanistic understanding of processes, based upon oral immediate release (IR) tablet platform technologies and a roller compaction (RC) dry granulation process.

20.2 Overview of ICH Q8(R2) Pharmaceutical Development

ICH Q8(R2) [11] provides guidance on the contents of the *Pharmaceutical Development* section in regulatory submissions, that is, new drug applications (NDAs) or marketing authorization applications (MAAs). That is section 3.2.P.2 in module 3 of the common technical document (CTD) [18]. The guideline describes the concepts of pharmaceutical development, which "provides scientific understanding to support the establishment of the design space, specifications, and manufacturing controls" and further brings up the concept of QbD by stating that "quality cannot be tested into products; i.e. quality should be built in by design." The guideline thereby proposes a new approach both in pharmaceutical development and subsequently in QC within the commercial manufacturing arena, as the development process is now specifically designed to support it. Pharmaceutical companies are therefore encouraged to apply a systematic, scientific approach to drug development. This is not unique to ICH Q8 as a similar approach is described for drug substance in ICH Q11 [14].

20.2.1 Quality by Testing (QbT) or by Design (QbD)

No matter what the approach taken, the intention of pharmaceutical development is to provide a commercial drug product, which is designed to meet the patients' needs and the intended product performance. The strategies for this activity will vary from company to company and from product to product based upon a number of factors, including the compound's properties, intended target product profile (TPP), previous experience, available equipment, and so forth. The ICH Q8(R2) [11] guideline offers an empirical or a systematic approach to product development, or a combination of both. The systematic approach is normally defined as QbD and could, according to the guideline, include incorporation of prior knowledge, DoE, PAT tools, quality risk management (QRM) [11], and knowledge management throughout the life cycle of the product (see also chapters on ICH Q9 [12] and Q10 [13]). The guideline opens up a new way of approaching product development understanding from a regulatory perspective. Therefore, both strategies, that is, empirical and systematic, are important options to consider in making a rational choice for the development strategy.

20.2.1.1 Quality by Testing (QbT)

Empirical product development, also termed quality by testing (QbT), has historically been the sole regulatory paradigm. Simplistically, this strategy ensures product quality through comprehensive raw material testing, drug substance specifications, fixed drug product manufacturing process, in-process controls, and, in particular, end-product testing [19]. The philosophy behind this strategy is that if all elements are standardized, the process should run consistently throughout its life cycle. The success of this approach, however, implies that the inherent variability in the process is controlled by end-product testing. All relevant sources of variability are routinely monitored, and materials that are out of specification are rejected prior to manufacturing, that is, the process is under complete control.

The quality of the raw materials and the drug substance is monitored by testing according to the approved specifications or pharmacopoeial quality standards, that is, European Pharmacopoeia (Ph. Eur.) or United States Pharmacopeia (USP). If the materials fulfill the predefined criteria, they can be used in the drug product. During production, in-process controls are performed such as uniformity of mass, tablet hardness, friability, disintegration, etc., in accordance with the approved regulatory file. Changes are not permitted without filing supplements to the relevant agencies [8]. For a product sold in multiple countries, post-submission changes can become a significant challenge given the different regulatory practices. Finally, the finished products are tested for quality by assessing if they meet the manufacturer's regulatory approved specification, which for an IR oral solid dosage form includes

appearance, identity, assay, impurity testing, content uniformity (CU), and *in vitro* dissolution testing. If there is a specification failure, the batch is discarded with the economic risk and loss absorbed by the manufacturer. However, this final release test in itself does not necessarily provide information on the root cause of the issue; the manufacturer should investigate the cause of the deviation and initiate preventive actions to ensure the same deviation does not reoccur, that is, corrective and preventative action (CAPA) [13]. If the original specification was deemed to be unnecessarily restrictive, the manufacturer has the option to justify that proposed changes have no impact on patient efficacy and safety and request regulatory flexibility, such as approving a less restrictive specification. This combination of fixed manufacturing steps and extensive testing is what ensures quality for many of the legacy products in the pharmaceutical industry.

During product development the main focus is on identifying a formulation and a process that delivers consistent product quality within the predefined specifications, over the entire shelf life. Specifications for the active pharmaceutical ingredient (API), for example, particle size, are often derived using historical data from batches produced during development. Using a risk- and science-based approach, it should be evident that an empirical development-based approach may be sufficient to ensure acceptable product quality. For instance, for a simple drug product process, for example, an oral solution, where the process and scale-up is relatively simple or a product intended for a more limited market, this development and quality paradigm may be the best option from a resource point of view rather than the significantly more resource-intensive QbD strategy. As ICH Q8(R2) [11] allows for continued product development according to this paradigm, an assessment of QbD versus QbT should be undertaken at the start of any new product development activity.

20.2.1.2 Quality by Design (QbD)

The ICH Q6A [20], ICH Q11 [14], and ICH Q8(R2) [11] guidelines define quality as "the suitability of either a drug substance or a drug product for its intended use. This term includes such attributes as the identity, strength, and purity." This in itself does not designate the strategies for achieving design and control of the API or drug product. However, these guidelines also provide additional definitions, which can accommodate both QbT and QbD strategies, but the latter approach is more extensively described in the annex to the ICH Q8(R2) [11] guideline. QbD is a systematic, scientific, risk-based, pragmatic, and proactive approach to product development with special emphasis on product and process understanding with associated control strategies. The development starts with the initial quality target product profile (QTPP), which is required to identify quality critical characteristics from the patient's perspective. This information must then be translated into critical quality

attributes (CQAs) that the final product should fulfill combined with the establishment of critical process parameters (CPPs) to consistently produce a product with the desired characteristics [19]. This requires establishing a relationship between formulation and manufacturing process variables and any potential input variability (including APIs and raw materials) and the resultant product characteristics. This can then be used to implement a flexible and robust manufacturing process, which can adapt and produce a consistent product throughout the product's life cycle. This is described in the next section.

Ideally in a QbD paradigm, pharmaceutical quality is assured by understanding and controlling formulation and manufacturing variables, and the end-product testing is only conducted to confirm the quality of the product. Specifications should therefore be defined based upon their relevance to *in vivo* performance, for example, dissolution, and variations within the defined and approved formulation and process "design space." In theory, any changes made within this design space should deliver a consistent product quality and are not subject to regulatory approval. A design space is however not necessarily a required outcome from a QbD filing, as the effort of establishing process understanding should be commensurate with the complexity and risk of the process and the product [21].

For a pharmaceutical drug product, such as an IR tablet produced by wet granulation, a large number of raw material and processing variables will exist. Consequently, getting to a required level of understanding that provides sufficient insight into all of the product's CQAs may be overwhelming. As a simple example, figure 1 of the ICH Q8 annex shows a two-factor response surface. Modeling a nonlinear response surface would require an optimization design (e.g., a central composite face (CCF), with 11 experiments/batches if three center points were to be included). During early formulation development, such a design is often unrealistic to conduct due to limited API supply (often restricted by API route optimization, in parallel to the pharmaceutical development activities). One way to mitigate this challenge is to acquire (at least in part) formulation and process understanding in advance and prior to actual formulation development of any new chemical entity (NCE). This can be achieved by working with established platform technologies and applying *prior knowledge*. High priority projects and resource-intensive projects where prior knowledge is available should therefore be considered for a QbD path.

20.2.2 Pharmaceutical Development Described in the Regulatory Submission

The two development paradigms (QbT and QbD) described in the previous section contain many of the same elements, which are briefly described in the present section, while the next section focuses specifically on those elements important to a QbD approach. Ultimately the supporting development

activities end up in a regulatory file, which should describe the knowledge obtained to support the selected dosage form and ensure the formulation and manufacturing process are "fit for purpose." According to the ICH Q8(R2) [11] guideline, "at minimum those aspects of drug substance, excipients, container closure systems, and manufacturing processes that are critical to product quality should be determined and control strategies justified," where critical is defined as any variation impacting on the drug product quality.

The components in the drug product (i.e., the formulation), comprising of defined quantities of API and excipients, should be characterized and described in section 3.2.P.2 of the CTD, with special emphasis on the relative importance to the drug product. For the API this should include a physiochemical and microbiological description of the properties, which may influence the performance of the drug product or its manufacturability. Examples could include hygroscopicity, particle size, salt form, polymorphism, solubility, pH solubility profile, transmembrane permeability, assessment of the biopharmaceutical classification system (BCS) category, etc. For the defined excipients, their relative concentrations within the formulation and their potential influence on product performance (e.g., an antioxidant's ability to improve oxidative stability or a preservative systems' ability to inhibit microbiological growth) and/or manufacturing should be discussed relative to their intended function. Further, the excipients' ability to maintain their function throughout the products' shelf life should be suitably demonstrated, for example, antioxidants, preservatives, and disintegrants. Excipient selection, following compatibility assessments, is the obvious first steps in any rational drug development; however, there are no general well-defined principles for testing and selecting of suitable excipients. This is in spite of the fact that unfavorable combinations of drugs and excipients may alter both the stability and the bioavailability of the drug in the formulation [22, 23]. Excipient ranges also need to be justified and relevant *in vitro* and *in vivo* studies discussed relative to the formulation in order to link clinical to commercial formulations. This part of the development process often applies in-house methodologies, but subsequently the final judgment is made based on formal stability studies [24].

The description of the manufacturing process should include a range of information related to the process and those CPPs identified during the development process that affect product quality. Not only equipment suitability should be discussed, but also the process development history to support and form the basis of the process control requirements, in-process controls, and general control strategy. Collection of data arising at different manufacturing scales, that is, process scale-up, should be included to facilitate an increased process understanding.

Finally, there is a need to describe the rationale behind the selection of container closure system(s) for the final product, including justification of both primary and secondary packagings. These considerations should include

intended use, transportation, storage, and related suitability of the container. Further, if dosing devices are used (e.g., pen injection devices, dry powder inhalers (DPIs), etc.), there should be a demonstration of their suitability, together with a demonstration of their ability to reproducibly and accurately deliver the intended dose. This should be done "under testing conditions which…simulate the use of the product," that is, through human factor studies conducted in patients and/or health care professionals, depending on the intended use of the device [25] or the ISO standard 62366 [26], if intended for the EMA region.

The previous description only covers some of the aspects described in the guideline, and it is therefore recommended to fully consult the guideline for further details. If additional data provides insight, supporting a selection decision related to the drug product or its manufacturing process, it should be presented and discussed in the file to allow the reviewer to gain a better insight and understanding of the work process that led to the final drug product.

20.2.3 Quality by Design Elements in Pharmaceutical Development

The main elements in QbD include:

- Definition of a QTPP
- Identification of the potential CQAs of the drug product
- Determination of the product design including identification of critical material attributes (CMAs) for the excipients and CQAs for the API
- Selection of an appropriate manufacturing process taking the physicochemical properties of the drug substance into consideration
- Identification of CPPs and a thorough understanding of scale-up principles linking CMAs and CPPs to CQAs, through, for example, prior knowledge, experimentation, and risk assessment
- Definition of a control strategy, including specifications for drug substance, excipients, and drug product as well as controls for the manufacturing process, which may include a design space and in-process monitoring and real-time release testing (RTRT), that is, potential use of PAT [7]
- Consideration of process capability and continual improvements making the process truly iterative

Empirical development includes many of the same elements; however, it may omit the systematic investigations and focus on defining an optimal product in predefined ranges.

20.2.3.1 Quality Target Product Profile

The QTPP is a summary of the quality characteristics of a drug product to ensure the desired quality, reflecting the safety, efficacy, and use of the drug product.

A QTPP for an IR tablet formulation can be very simple and similar from product to product (i.e., generic), but the clinical literature may provide some insight into dosing intervals and release profiles that are important to capture in the QTPP. Other more complicated formulations, such as powder for inhalation or subcutaneous injections of biologicals, may require additional knowledge to construct the QTTP.

Many companies have platform technologies (see following section), that is, dry or wet granulation, and associated prior knowledge from other products. Basic work may be used to ensure a more effective and efficient development process. Based upon these initial considerations and sound scientific approaches (including supporting scientific literature), the CQAs for the product should be identified. CQAs are more quantitative than the QTPP and are defined according to the guideline as "a physical, chemical, biological or microbiological property or a characteristic that should be within an appropriate limit, range or distribution to ensure the desired product quality," which could include elements such as assay, impurities, etc., and physical attributes such as color, shape, size, etc. The initial list of CQAs can be prioritized based upon scientific judgment and on the relative risk of harming the patient. Given the iterative nature of product and process development, the QTPPs and CQAs may be refined from the initial list during the development as more information becomes available. For instance, CQAs of the product may be linked to the specific variables of the manufacturing process, that is, process parameters and material attributes and variations. A functional relationship between those parameters may be established using, for example, DoE and multivariate modeling. The CQAs of the drug product are typically included in the final product specification or functionally related to other tests included on the final drug product specification (i.e., dissolution testing in lieu of particle size distribution (PSD)). CQAs should therefore be both science- and experience-based and evaluated with respect to impact on the patient. Combining the severity evaluation with risk analysis of the suggested manufacturing process can therefore be a good prioritization tool, helping the formulation scientist to focus on the most critical activities to obtain a well-understood process, resulting in appropriate product quality [12].

20.2.3.2 Formulation Development

The key objective of the formulation development and thereby product design is to develop a robust product, designed both to meet the QTPP and the patients' needs. The key elements under a QbD paradigm are to a large extent the same as described in the historical approach to pharmaceutical development (described previously), however, with increased focus on a better understanding of interrelationships between critical attributes and parameters. Studies to optimize the formulation are essential and should ensure a robust

formulation that is not on the "edge of failure." Without these studies, the formulation is more likely to be suboptimal as changes in the formulation itself or the raw material properties may have an adverse impact on the quality of the drug product [27, 28]. The formulation optimization work should:

- Ensure a robust formulation including the establishment of the relationships between product's CQAs and input variables like CMAs (excipient), drug substance's CQAs, and CPPs
- Deliver a scientific basis for the control strategies for drug substance, excipients, and drug product

Post-approval variations are very likely to occur in the input materials. Therefore, the CMAs are important to investigate to ensure future robustness of the formulation. This is particularly true where the functionality of the excipients affects those attributes critical to the patient, such as batch-to-batch variations of HPMC in modified release tablet formulations [29]. This work should be scientifically based and, together with a risk assessment, should define experiments used for clarification of the risk. Further, it should be used to develop models to determine if the risk is truly critical to the quality of the product. CMAs should be defined as an appropriate limit or range required to obtain the desired quality and should be based upon the data from the optimization work and also the formulator's expertise. These kinds of evaluations could form part of the design spaces and are useful from a regulatory perspective, as they can provide a scientific basis for noncritical variations that should not be subject to regulatory approval in future planned or unplanned process changes [21].

20.2.3.3 Process Design

A pharmaceutical manufacturing process usually consists of a series of unit operations designed to produce the final product. This can be in batch mode or—less frequently—linked together to provide a continuous manufacturing process. The individual subprocesses that make up the entire manufacturing process comprise a variety of different process parameters. These are typically referred to as operating parameters (e.g., impeller speed in high shear (HS) mixer granulation, binder liquid flow rate, etc.) and/or process variables like temperature and pressure. As with a formulation parameter, a process parameter is critical when its variability has an impact on the final product quality and should therefore be monitored or controlled.

The effect of the variation in the process parameters and material attributes is investigated during process screening, optimization, and robustness studies, typically using DoE approaches. The analysis of these studies should identify the CPPs and CMAs, the related CQAs, and the critical relationship between these input variables. The steps to establish process understanding

are essentially the same as those for product understanding and could include the following:

- Identify known process parameters that could impact the performance of the process
- Use risk assessment [12], pre-experiments, scientific knowledge, and prior knowledge to identify potential critical parameters and attributes
- Establish appropriate levels or ranges of these potential critical parameters and attributes
- Define and conduct experiments using DoE where relevant to map the relationships between critical parameters and attributes
- Analyze data and develop a model capable of linking CMAs and CPPs to CQAs and the impact of scale (if possible)
- Develop a control strategy and if relevant a design space

Conducting full-scale DoE experiments introduces a significant economic burden, hence the best strategy is to establish formulation and process understanding at experimental scale and confirm scalability through appropriate experiments at a larger scale. A rational approach is to aim for a suitable level of process or optimally a mechanistic understanding and to apply innovative equipment design (see later sections).

20.2.3.4 Control Strategy

All of the knowledge accumulated during the development of the drug product should form the basis for establishing a control strategy. Under the QbT paradigm, the control strategy would involve rigid control of materials, process parameters, and end-product testing. Due to the limited understanding of interrelationships between material, formulation, and process, any change would require regulatory approval.

When the formulation is developed under a QbD paradigm, implementation of RTRT is an option. RTRT is an outcome of a control strategy whereby product quality is assured through a combination of process information and input or in-process material attribute measurements performed during manufacturing—in lieu of traditional off-line end-product testing. In situations where the input materials can be monitored, process parameters may be automatically adjusted within an established design space, based upon material and process understanding, to assure that the identified CQAs are within the defined acceptance criteria.

A control strategy can include the following:

- Control of input materials, such as excipients, drug substance, and packaging materials based upon generated knowledge of their impact on the process and the product quality
- Product specification(s) [20]

- Control for intermediates, for example, PSD of granulate, which may impact processing or quality for later process steps or the final product
- In-process or RTRT
- A monitoring program to verify the ongoing capability of the mathematical models that have been developed

The control strategy thereby points backward into the development of the CQAs, which in turn have been based upon thorough scientific work.

20.2.3.5 Continual Improvement

Both ICH Q8(R2) [11] and ICH Q10 [13] describe continual improvement initiatives, which are applicable to all products independent of the development strategy documented in the regulatory file. Continual improvements are not only those set of activities conducted to ensure the ability to meet market requirements but also a scientific approach to capture some of the variations in materials observed over time. This requires collection and analysis of the batches routinely produced and trend analysis of any potential variation to identify cause/effect relationships.

20.3 Utilizing Prior Knowledge to Facilitate QbD in Formulation Development

20.3.1 Introducing the Platform Concept

Identifying the optimal composition of a solid dosage form and choosing the most effective process technology for subsequent manufacturing are a multidisciplinary task. The relationship between attributes of the active ingredient, excipient functionality, and processing environment should be understood in order to provide a robust formulation and manufacturing process. As described in previous sections, initial risk assessment of potential critical parameters and attributes, designed experiments, and timely (and proper) application of analytical tools (either PAT [7] or offline) are key tools in establishing this relationship. While correct application of QbD principles delivers assurance of product quality, carrying out a full QbD program on an NCE does not necessarily provide an effective development workflow. QbD may necessitate the need to investigate numerous input variables. Risk ranking [12] is therefore a useful tool to filter the variables and thereby ensures appropriate focus on those parameters most likely to impact process performance and/or product quality. This needs to be assessed in light of the guidance from FDA on process validation [30], which emphasizes how process understanding can only be fully achieved by linking information collected from early lab-scale development batches to commercial-scale batches. The challenges that the pharmaceutical industry is facing in relation to the QbD paradigm can be illustrated by the

"project management triangle," showing the three constraints of product quality: scope, resources, and time (see Figure 20.1). In a QbT paradigm, a request for enhanced product quality is typically achieved by the tightening control of input materials, in-process parameter settings, or end-product testing, with the resultant economic consequences for the manufacturer. In contrast, in a QbD paradigm a request for enhanced product quality can only be achieved by increasing the scope, resources, and/or time spent on formulation development, and to a much lesser extent on process scalability. In pharmaceutical development, *time* is typically not a variable parameter as it is fixed according to the timelines governing the duration of the pivotal clinical trials. Also, due to the already high cost of clinical trials as well as the relatively high risk carried in the development of an NCE, it is often not realistic to increase the number of *resources* consumed in formulation development. This only leaves an increase in *scope* as a viable option to provide a QbD-based formulation and process development and the improved assurance of product quality that follows. An innovative way to increase scope is to work with *platform technologies* and the *prior knowledge* embedded in these Refs. [31–33] as means to acquire parts of the formulation/process understanding prior to actual NCE formulation development. Prior knowledge is the backbone of every platform technology, and it is important to define, especially since it is a widely used risk mitigation term in regulatory submissions. According to Yu et al. [4], the word *prior* not only means previous but also associates with ownership and confidentiality not available to the general public. In other words, information regarded as prior knowledge is gained through experience generated within a given company and has to be documented. In contrast, information gained through education and scientific literature is termed *public* knowledge. Therefore, although technology platform is a widely used term in many companies, experience gained *in-house* on that particular technology is a prerequisite for it to be truly adopted as a platform technology. Platform

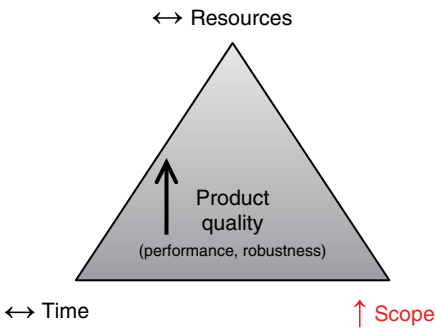


Figure 20.1 The *project management triangle* and the three constraints on a project deliverable impacting on product quality (resource, time, and scope).

technologies vary greatly in their application across different industries, but they all serve the same purpose: to streamline the development of future products by drawing upon previously established knowledge gained on *generic* aspects of the product. The generic parts may also be regarded as the attributes that are shared between previous and new products. Examples are modular designs [34] generated for a variety of products such as cars, power tools, and toys, an example of the latter being LEGO® and their wheel platform used to lower manufacturing costs of new LEGO® vehicles in development [35].

Pharmaceutical technology platforms can therefore improve the effectiveness of product development by ensuring that the primary focus is on understanding CQAs that are heavily dependent on the NCE rather than properties governed by the functionality of the excipients (which can be covered by prior knowledge). Improved effectiveness in development may also reduce costs due to more rational/constrained choices being made regarding formulation composition and, in particular, manufacturing process selection (dry vs. wet granulation and the number of unit operations). Lundbeck has chosen to define a platform technology according to formulation type (e.g., immediate or modified release), formulation elements (excipient choice, composition), and applied process technology elements, such as direct compression (DC), RC, fluidized bed (FB) granulation and high shear wet granulation (HSG). Critically, the in-house experience gained on these platform technologies is transferable and focuses on understanding the interplay between formulation and process technology on a set of *generic* product properties assessed by relevant analytical tools (e.g., PAT [7]) (see Figure 20.2). It should be emphasized that the platform-based approach is not suitable for all pharmaceutical products. When APIs have a relatively high potency, they are typically present in low concentration in the tablet product (e.g., <10% w/w). In these cases, many of the resultant tablet properties depend primarily on excipient functionality (e.g., flow properties, compression characteristics, etc.) as well as physical parameters of the excipient and API (particle size and morphology), which affect the distribution between API and excipient in the blend/tablet. A large part of the process understanding may therefore be acquired in advance and

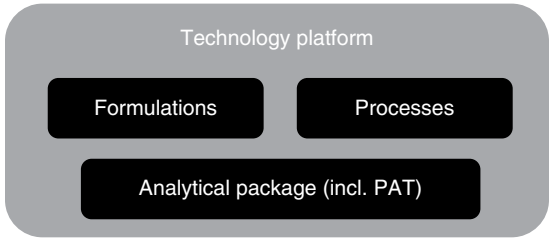


Figure 20.2 Elements of a pharmaceutical technology platform.

described in a more general context by distinguishing between API-specific product properties and excipient-related properties (see Figure 20.3). Companies working with products requiring high API loadings would have to include additional relevant API properties (e.g., flow properties, compression properties, solubility, etc.) to fully understand the application range of the desired platform.

Once the platform has been constructed, typically using a company specific model API, it can be used proactively to predict optimal starting points for the formulation development of an NCE as outlined in Figure 20.4. Lundbeck's approach to the construction and application of our IR platform for RC technology is given in a later section. The broader context in which a platform technology/prior knowledge is applied is illustrated in Figure 20.4.

Building on input from early feasibility assessments and compatibility studies, the formulation and manufacturing process is agreed. These choices are based on scientific insight acquired from a combination of in-house experience (i.e., prior knowledge) as well as publicly available literature. Selecting the most effective manufacturing process is an important activity, as this process will typically not be changed throughout the products' entire life cycle. Once regulatory approval is achieved, it is no longer realistic to alter the manufacturing process. For example, changing a costly FB process to a cheaper DC process requires a full development package, including a new file, and it may require bridging bioequivalence (BE) studies (dependent on biopharmaceutical properties of API). However, formulators are sometimes prone to selecting the

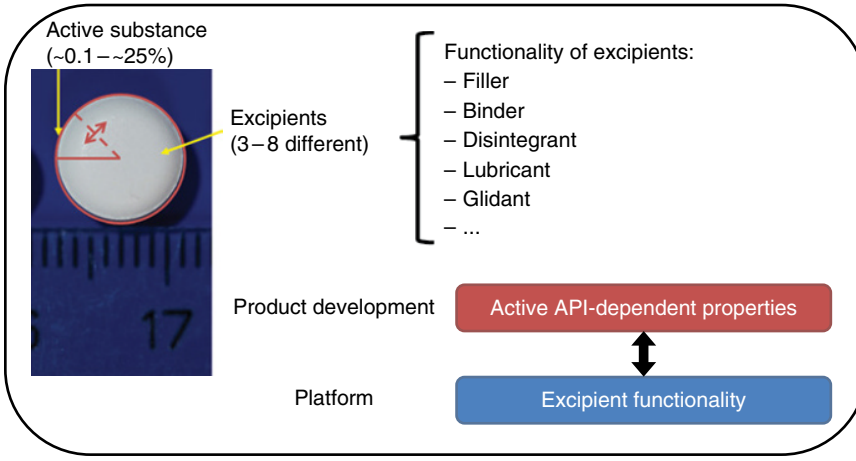


Figure 20.3 Simplification of a platform technology. Knowledge captured in the platform covers quality attributes that are primarily affected by the excipients. API dependent properties (e.g., dissolution, stability) are explored in more detail as a part of an actual NCE product development.

process technology with which they are most familiar, thereby neglecting more cost-effective alternatives that may provide either the same or enhanced drug product quality. Again this stresses the multidisciplinary nature of such crucial decisions and the importance of sound scientific judgment.

Throughout the various steps outlined in Figure 20.5, it is recommended to apply an appropriate level of process understanding to support the decision-making. For unit operations likely to have a high impact on the variability of the product quality, it could therefore be beneficial to aim for first-principles models or *mechanistic understanding* whenever feasible. First-principles models are based on theoretical understanding and therefore initially do not require

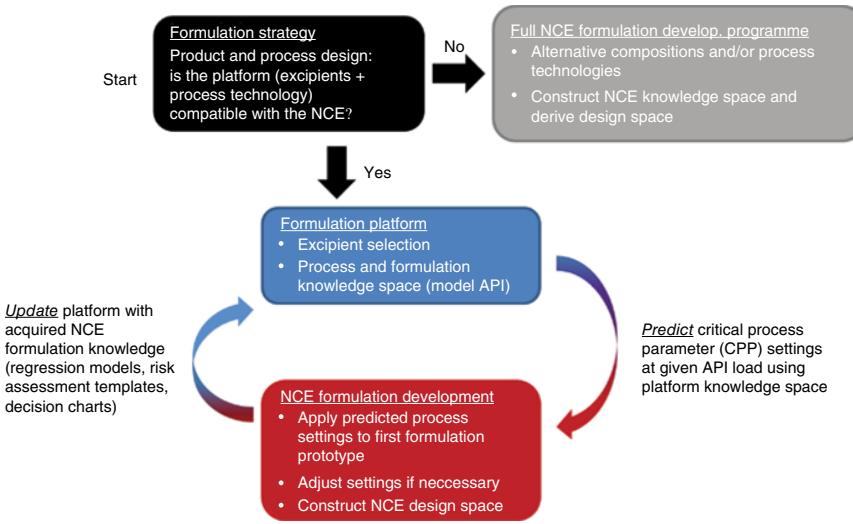

Start
Formulation strategy
Product and process design: is the platform (excipients + process technology) compatible with the NCE?
No
Full NCE formulation develop. programme
• Alternative compositions and/or process technologies
• Construct NCE knowledge space and derive design space
Yes
Formulation platform
• Excipient selection
• Process and formulation knowledge space (model API)
Update platform with acquired NCE formulation knowledge (regression models, risk assessment templates, decision charts)
Predict critical process parameter (CPP) settings at given API load using platform knowledge space
NCE formulation development
• Apply predicted process settings to first formulation prototype
• Adjust settings if neccessary
• Construct NCE design space

Figure 20.4 Illustration of how the formulation platform is applied to NCE formulation development. Output from the formulation strategy triggers application of the platform.

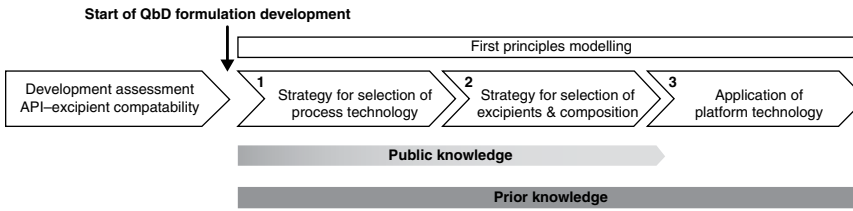


Figure 20.5 Illustration of the stages during QbD formulation development, leading up to a final formulation that delivers the desired QTTP and is technically feasible to manufacture. Public as well as prior knowledge is utilized in the strategies behind the selection of process technology and excipients, while a platform technology is based solely on in-house practical experience, that is, prior knowledge. First-principles models are incorporated throughout all three stages.

experiments, and they can aid for example in the setting of API particle size specifications in relation to DC products but could also be used to translate CPPs for an FB product into parameters that are better correlated to granule formation. As with platform technologies, first-principles models are used to improve the overall effectiveness of the formulation development process.

20.3.2 Rational Selection of Excipients, Composition, and Process Technology

It is not unusual that the choice of both formulation and process technology is based on empirical considerations on what historically has been shown to work for other products. However, this is not always a rational or effective approach to utilize. In contrast, the QbD paradigm requires a scientific and risk-based product development strategy in which the selection of excipients, composition, and process technology are scientifically justified [11]. Rational selection of excipients for the tablet formulation is naturally interlinked with the selection of technology platform [29]. The rational selection criteria described later is based on the assumption that the IR tablet has a relatively low strength (e.g., <100 mg) using a BCS [36] class I API.

20.3.2.1 Selection of Process Technology

The following section exemplifies how rational selection criteria for choosing process technology can be defined based on public and prior knowledge (see the case studies described later in this chapter on RC products). In addition, the proposal for a Manufacturing Classification System (MCS) [37] provides additional insight, including the use of appropriate expert systems.

General technical requirements for an IR tablet product are specified in the appropriate QTPP (see Table 20.1). The selection of a tablet composition and process conditions should be optimized to fulfill these generic criteria.

In the early development phase, the dosage form is often a capsule since the dose range has not been fully established and the capsule allows greater freedom to adjust the dose. After clinical proof of concept (POC), a tablet formulation is often preferred as the final marketed product. At that stage, selection of a final formulation and manufacturing process is agreed and typically remains fixed throughout the development process, in order to avoid any unwanted BE studies. The choice of process technology is dependent on dose, stability, and physicochemical properties of the API, for example, PSD and particle morphology. A prioritization of process technology should be based on a risk mitigation strategy and is a compromise between minimizing process complexity and the ability to satisfy process and product requirements relating mainly to API properties. The primary process technologies for tablet manufacturing are DC, RC, and WG methods (see Table 20.2). In order to reduce the number of CPPs and scale-up variables, the ideal choice would be DC due to the decreased

Table 20.1 Technical specifications for an immediate release tablet as part of QTPP.

Tablet properties	Quantitative specification	Qualitative specification
Mass variation	<1%	
Disintegration time	<3 min	Relative robustness against compression force
Dissolution	>85% release in 60 min	Relative robustness against compression force
Friability	<0.2%	No edge fractures
Appearance	Surface roughness spec.	No capping or adhesion to punches observed
Hardness	>40–80 N (size dependent)	Approximately linear correlated to compression force in a broad range
Content uniformity	RSD < 4% compensated for mass variation. Calculated on basis of assay on tablets sampled regularly during tableting ($n > 20$)	No trend in assay during tableting (no segregation)

process complexity/cost compared with RC and WG. However, DC requires strict control over the particle size of the API in order to obtain a stable blend with appropriate flow and compaction properties for compression on a high speed tableting machine while satisfying the requirement of dose homogeneity during tableting. For lower dose products, where the API loading is <1–2% of the compression weight, a stable blend can be obtained by micronized API and using fillers with larger "carrier" particle size, for example, microcrystalline cellulose (MCC) in an HS mixer. In this case, stable non-segregating blends are formed, where the tablet compactibility is primarily determined by the principal excipient, that is, the filler (e.g., MCC). At larger relative doses, the smaller particle size fraction of the API may become critical to dose homogeneity, segregation during tableting, adhesion to punches, and compactibility of the blend. Since it may be difficult to control the PSD during API production, particle size is often indirectly controlled by micronization by mechanical milling or jet air milling. However, this results in increased cohesiveness of the API, making it difficult to handle using a DC process. Therefore, despite DC being the most obvious process technology, it is often necessary to choose process technologies that are less sensitive to variation in PSD (c.f. Table 20.2).

Historically, WG by HS granulation or FB granulation was the most common processes technologies used in the manufacturing of IR tablets. However, the trend over the last decade has seen increased usage of RC as this is a cost-effective low complexity process, easy to develop, and scale-up, which shows limited impact of particle size of the drug substance across a relatively

Table 20.2 Process technology for granulation or tablet manufacturing and potential parameters to be considered in-process selection.

Process type	Degree of process complexity and number of scale-up variables	Degree of sensitivity to API-PSD	Risk of product segregation—dose inhomogeneity
Direct compression (DC)	*	****	Especially if pneumatic transport of the blend is involved****
Roller compaction, dry granulation, (RC)	**	***	Larger content of fines due to milling of ribbons**
Fluidized bed wet granulation (FBG)	***	**	Narrow granule size distribution*
High-shear mixer wet granulation (HSG)	****	*	Narrow granule size distribution*

**** Highest level of process complexity, sensitivity to API-PSD, or risk of product segregation.

broad dose range (see later section). For NCEs, HSG should only be used for highly cohesive drug substances that require densification during granulation and in cases where other process technologies have failed. HSG is a difficult technology as scale-up parameters are highly qualitative and cannot be based on a generic platform. FBG is far better mechanistically understood, and scale-up can be based on the first-principles model (FPM). In contrast to RC, and especially DC, the risk of granule segregation that impacts on dose homogeneity of the tablets can be minimized by "interlocking" the drug particles into granules characterized by a relative narrow PSD obtained by FBG. Segregation of an FBG product is only a risk factor for dose homogeneity if the drug substance is not homogenously distributed between the different granule size fractions (which should be investigated for all granulated products) or if a larger proportion of an extra-granular filler phase is used prior to compression. In addition, WG also introduces a further risk of destabilizing the API (e.g., hydrolysis, change in crystal form, or hydrate formation) [29], which again favors DC and RC as preferred process technologies.

A general guide for selection of the appropriate process technology, based on API particle size, is shown in Figure 20.6. The guide is devised from experience obtained during the establishment of our technology platforms for DC, RC, and FBG. The categorization described is simplified, since API particle morphology is not taken into account, which may affect the manufacturability significantly (e.g., needle-shaped vs. platelike crystals).

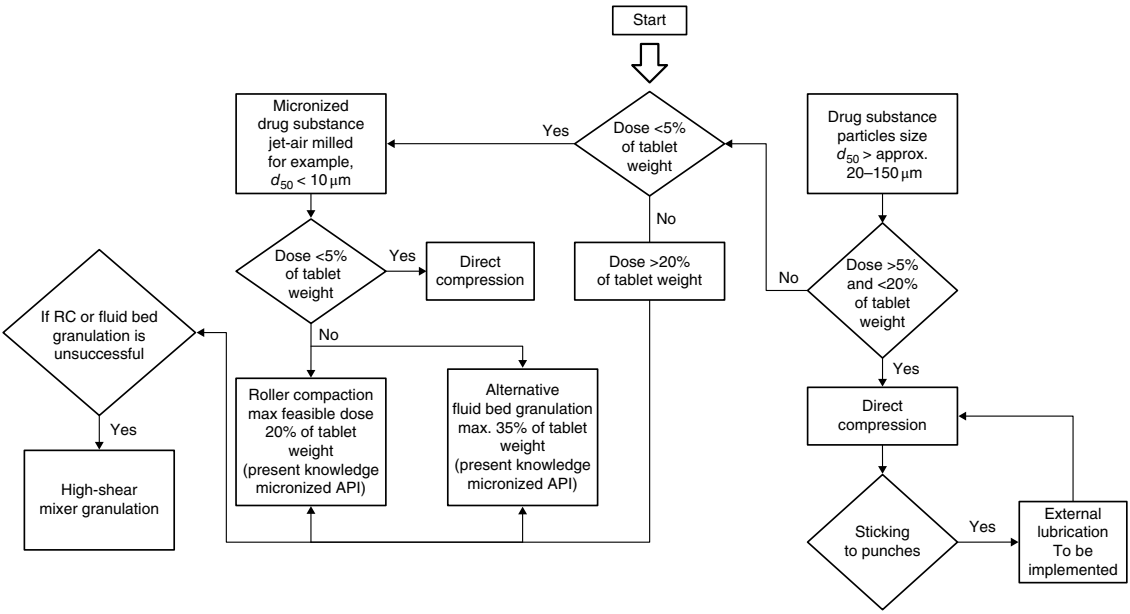

Start
Dose <5% of tablet weight
Yes
Micronized drug substance jet-air milled for example, d_{50} < 10 μm
Dose <5% of tablet weight
Yes
Direct compression
No
Roller compaction max feasible dose 20% of tablet weight (present knowledge micronized API)
Alternative fluid bed granulation max. 35% of tablet weight (present knowledge micronized API)
If RC or fluid bed granulation is unsuccessful
Yes
High-shear mixer granulation
No
Dose >20% of tablet weight
Drug substance particles size d_{50} > approx. 20–150 μm
No
Dose >5% and <20% of tablet weight
Yes
Direct compression
Sticking to punches
Yes
External lubrication To be implemented

Figure 20.6 Selection criteria of process technology based on median volume particle size (d_{50}) of the drug substance (regular shaped crystals) and the relative tablet dose % w/w.

20.3.2.2 Selection of Excipients and Formulation Composition

The overall formulation rationale should adhere to the principle of keeping it simple and only introducing excipients that can be justified [29] and in a concentration required to satisfy the QTPP and those critical in-process parameters or CQAs. Based on this strategy we have selected and evaluated some preferred excipients for IR-tablet formulations. The following examples describe the justification behind selection of some excipients and how it aligns to DC and RC as generic technology platforms.

Selection of a tablet filler is for practical reasons constrained to three different excipients: MCC, mannitol, and lactose. MCC is known to possess excellent compressibility and compactibility properties. It takes up moisture at elevated humidity, but the water is bound internally into the polymer and does not necessarily introduce additional risks of product instability. Further, the content of peroxides is low, so the excipient is a good choice in cases where oxidative degradation of the API is a known risk.

Lactose is also commonly used as a tablet filler or diluent. However, it is incompatible with primary and secondary amines, via the Maillard reaction [38], which can cause product instability. In contrast to lactose, mannitol is a nonreducing sugar and does not react with amines and only takes up minimal amount of moisture at elevated humidity. Both lactose and mannitol do not contain residual peroxides.

Although it is not uncommon in the literature to rely on mixtures of fillers, for example, MCC and lactose, the biggest disadvantage of such an approach is the increased likelihood of API incompatibility. At Lundbeck, all platform formulations are therefore developed using a single filler and in some cases with a minimal amount of added binder to compensate for insufficient compression properties of the selected filler. Incompatibility between the API and any given filler, for example, lactose, can then be addressed by simply selecting an alternative excipient, for example, a MCC-based platform formulation.

We have built up a "knowledge space" for these three fillers by investigating how specific functionalities change with respect to different process technologies, for example, DC, RC, or FBG. This has resulted in some general guidelines on first choice of fillers, as well as binders, disintegrants, and lubricants, for any given process technology platform.

20.3.3 Applying First-Principles Models (FPMs) to Facilitate a Science-Driven Development Process

20.3.3.1 The Importance of First-Principles Models in Pharmaceutical Development

FPMs are mathematical models, which establish a quantitative relationship between process input and output. The models are based on fundamental engineering, physical, or chemical principles in contrast to empirical

mathematical or statistical correlations between input and output variables derived from historical data. Two examples of FPM are described later. These examples discuss the initial PSD specifications (a CMA) for an API focusing on (i) CU and (ii) dissolution, both highly relevant CQAs of the drug product. It is well recognized that API PSD specifications are challenging, especially in the early phases of product development where only limited numbers of API batches are available. Consequently, there are limited data available for establishing an empirical correlation between API PSD and CU or dissolution for the resultant drug product.

20.3.3.2 Example 1: Calculation of Optimal Blend Stages in Direct Compression

The effect of PSD on blend homogeneity is based on the assumption that a binary blend can be regarded as optimally blended when the two particle systems are randomly mixed on a statistical basis [39]. Further, the assumption is made that the particles do not interact physically and are free flowing, which may not always be the case. However, this model has in our experience been shown to be reliable based on a large number of different DC products demonstrating a satisfactory correlation to experimental CU data. This model has also shown to work with ternary mixtures of API and two fillers, by approximating the combined PSD for the fillers as an input variable, but it does not take into account the other minor components, for example, lubricants or disintegrants. The calculation of the optimal blend homogeneity (CU = $S_{A,rel}$%) related to the specific tablet weight (w) and tablet strength is defined by the equation

$$S_{A,rel}\% = 100\sqrt{\frac{P_B\left(P_B w_a + P_A w_b\right)}{P_A w}} \tag{20.1}$$

where w_a and w_b are the mean weight of single particles of API and filler(s) and P_A and P_B, are the relative contents of API and fillers, respectively, where $(P_A + P_B) = 1$. The mean weight w_a, w_b of a single particle of component a or b is calculated from the volume size distribution according to Eq. 20.2 using the volume PSD data obtained from laser diffraction measurements:

$$w_a = \frac{\pi}{6}\rho\sum_i v_i d_i^3 \tag{20.2}$$

where v_i is the particle volume of fraction i, d_i is the mean particle size of fraction i, and ρ is the density of the particles determined by a gas pycnometric method.

The model can be determined using Excel®, allowing for direct input of experimentally determined PSD data for API and filler(s) and particle densities.

Furthermore, different simulated API PSDs can be used as input parameters, and it can also be used for a sensitivity analysis of the CMAs based on different input API PSD data as inputs to the initial PSD specification (assuming a satisfactory theoretical CU output). It is essential that the FPM data are correlated to experimental CU data acquired during product development in order to adequately validate the model and provide optimal basis for setting the final API PSD specifications. One example of the correlation between FPM-based CU and experimentally determined CU (compensated for tablet mass variation) is shown in Figure 20.7. These data are based on a DC formulation of different strengths with different API PSD blended with one major tablet filler, that is, MCC. As can be seen in Figure 20.7, the FPM gives reasonable guidance for setting API PSD specifications. In this example, the mean particle size of the drug should be $d_{50} < 40$ μm to produce a satisfactory CU in the desired dose range.

20.3.3.3 Example 2: Effect of API Particle Size on Dissolution Behavior (*In Vitro*/*In Vivo*)

The effect of API PSD on dissolution rate and oral absorption is described in a simplified FPM by Hintz and Johnson [40]. The basic principle is to divide the PSD of the API into discrete particle size classes and calculate the dissolution rate from each single particle size class. The dissolution rate dX_i/dt for a specific particle size class with mean radius r_i containing N_i particles is determined by the Noyes–Whitney equation [41] where C_s is the solubility of the API in the dissolution media and C_v is the dissolved API concentration at a given dissolution time given by Eq. 20.3:

$$\frac{dX_i}{dt} = N_i 4\pi r_i^2 \mathrm{IDR} \frac{\left(C_s - C_v\right)}{C_s} \tag{20.3}$$

The intrinsic dissolution rate (IDR) (mg/cm^2/min) is determined from a well-defined compressed disc of the API under sink conditions. The summation of the dissolved amount of drug for all particle size classes as a function of time results in a theoretical dissolution curve in a specific media. The dissolution model is easily transferred to Excel with input of the API PSD determined experimentally by laser diffraction. Similarly, a sensitivity analysis can be applied to the simulated API PSD for setting the initial PSD specifications, if required. As for all FPMs it is important to validate the model against experimental dissolution data, eventually fitting the IDR to a more representative value for the system. The model can easily be adopted to include oral absorption data. Adding the absorption rate to the mass balance of Eq. 20.3 requires input by an estimated intestinal permeability constant using, for example, Caco-2 cell-based data. This model is highly simplified since variables such as *in vivo* changes in pH or permeability during intestinal passage are not taken

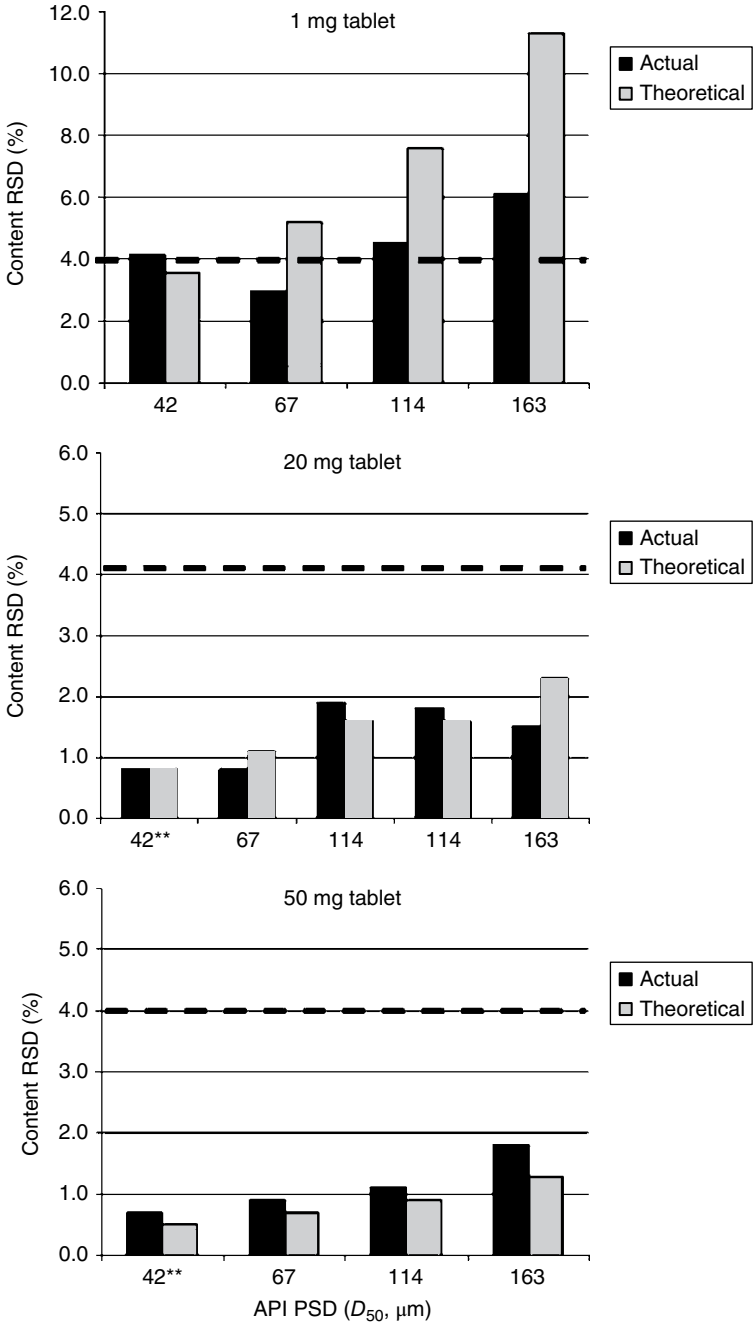


Figure 20.7 Variations in content of uniformity displayed by the relative standard deviation between tablet assays sampled over 100 min of total tableting time. Two tablets have been analyzed for content every 10 min. Maximum allowed content RSD is 4% indicated by a dashed line. **Punch-face adhesion observed. *X*-axis represents median volume particle size of the API.

into account, as well as pH-dependent API solubility or API precipitation. It is therefore highly important that an FPM on oral absorption is supported by other more sophisticated simulation model software, such as SimCyp or GastroPlus. However, the model can be considered as a starting point for the development process.

20.3.4 Platform Technologies: Roller Compaction (RC)

20.3.4.1 Introduction to Roller Compaction Technology

RC, also referred to as *dry granulation*, introduces an extra-compaction step into the process, in addition to tablet compression. The process stages of a roller compactor may roughly be split into (1) powder feeding to the compaction zone, (2) powder densification into ribbons (or flakes), and (3) milling/size reduction of ribbons into the final granule product [42] (see Figure 20.8).

RC is a continuous unit operation, and the primary "batch" may be split into several sub-batches and processed at various combinations of process parameters. This approach enables efficient formulation development as well as flexibility to move between various scales by adjusting either press roller speed or gap size. The latter approach should only be initiated when the impact on

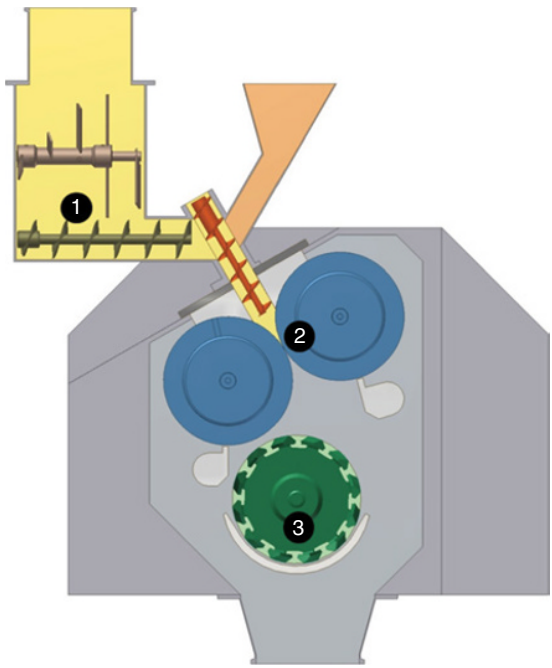


Figure 20.8 Illustration of a roller compactor (Gerteis model). 1, powder feeding; 2, compaction; 3, size reduction/milling (granulator).

granule quality is understood (see RC knowledge space). Alternatively, the process throughput/scale can be maintained, and capacity considerations can be addressed by using additional granulators in parallel (the so-called "parallel array" approach). CPPs are discussed in the following section. The work described in the following sections was performed on a Mini-Pactor (Gerteis Maschinen Process Engineering AG). More details on process settings can be found in Allesø et al. [33]

20.3.4.2 Platform Formulation Composition and Ranking of RC Parameters

It is advisable to select an API model substance that has a well-understood process history within the company. Both the model and test substances used in the current work are of similar molecular weight, that is, 400–500 g/mol, and their respective solubilities were aligned with the use of an IR solid dosage form.

Due to its ability to plastically deform, MCC is the first choice filler for dry granulation by RC. It is sometimes argued that a plastic deforming material should be mixed with a brittle one (e.g., lactose) in order to reduce work hardening effects [43]. The loss of re-compactibility of a plastic deforming material may, however, be overcome through the control of granule porosity via applied roll pressure. By keeping the ribbon porosity within a specified limit (typically in the range 20–40%), one ensures a sufficient number of inter-particulate bonding sites are available for subsequent tablet compression (see next section). The composition of the MCC-based platform formulation for RC is shown in Table 20.3. The API fill weight ranges from approximately 1–20% w/w and covers most of Lundbeck's existing products. However, within this range the mechanical properties of intermediate and final products are still dictated by the influence from the major filler component, that is, MCC and not the API.

After the formulation has been decided, attention should be paid to the CPPs (factors) and the quality attributes (critical in process attributes (CIPAs) and

Table 20.3 Composition of the MCC-based roller compaction formulation.

Phase	Composition	Range (% w/w)
Intra-granular	API (H. Lundbeck commercial substance)	1.1–20%
	Microcrystalline cellulose	Filler
	Croscarmellose sodium	3.0%
	Magnesium stearate	0.5%
Extra-granular	Magnesium stearate	0.5–1.5%

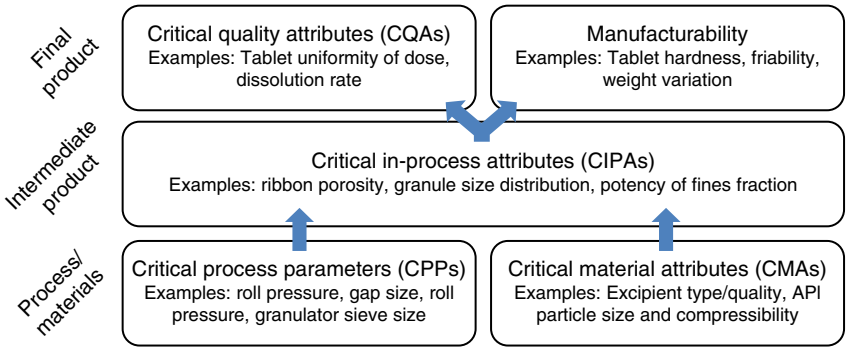

Final product
Critical quality attributes (CQAs)
Examples: Tablet uniformity of dose, dissolution rate
Manufacturability
Examples: Tablet hardness, friability, weight variation
Intermediate product
Critical in-process attributes (CIPAs)
Examples: ribbon porosity, granule size distribution, potency of fines fraction
Process/ materials
Critical process parameters (CPPs)
Examples: roll pressure, gap size, roll pressure, granulator sieve size
Critical material attributes (CMAs)
Examples: Excipient type/quality, API particle size and compressibility

Figure 20.9 Examples of critical input and output variables for roller compaction (RC) process and their mutual relationship.

CQA responses). The hierarchical links between the various input and output variables are illustrated in Figure 20.9. For the purpose of platform construction, it is important that the selected CIPAs or CQAs are primarily influenced by the excipients and to a much lesser extent the physicochemical properties of the API. Acceptable attributes are mechanical/technical properties of the granules/tablets, which could be a fraction of fines, tablet hardness, and others, as well as attributes related to the physical interaction between API and excipient, such as blend uniformity and tablet CU. In contrast, significantly less effort should be spent on the optimization of the dissolution profile of the model API, as later incorporation of an NCE will typically show a different profile (due to differences in solubility, wettability, sensitivity to lubrication, etc.). Thus, API dependent CQAs should be investigated during actual NCE formulation development and not as a part of platform construction.

A second issue pertains to the criticality ranking of process parameters. In the case of RC, there are potentially many process parameters to investigate, that is, tamp/feed auger speed, roll force/speed/type, gap size, granulator type/speed/angle, and sieve type/size. An exhaustive DoE study of all these variables is time consuming and not logical since several of these parameters have only negligible impact on the granule quality. In addition, the feed screw speed on high throughput compactors is automatically adjusted using a gap control feature. This gap control feature effectively reduces the number of process variables and thus workload during early formulation development [44]. In the case of ribbon porosity (CIPA), there are three major factors: roll force > gap size > roll speed. The points of impact of these parameters in the RC process are illustrated in Figure 20.10.

Roll force and gap size are known to exert the largest effect on ribbon porosity and thus the granule size distribution and, for the purpose of generating platform knowledge spaces, these are the prime focus of any DoE (see next section). In contrast, roll speed is most rationally investigated ad hoc during scale-up.

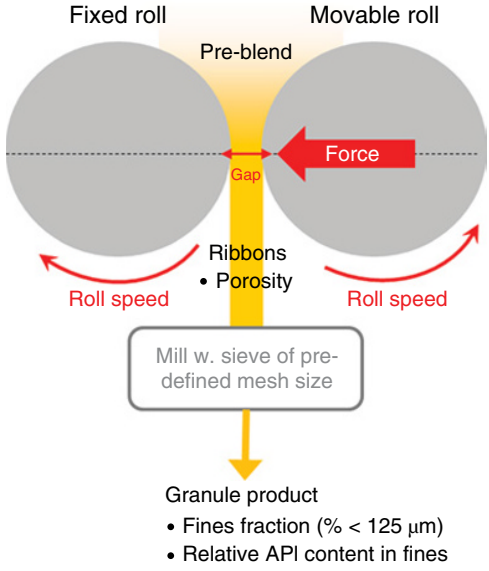


Figure 20.10 The principle of dry granulation using roller compaction (RC). Critical process parameters (CPP) and selected in-process quality attributes (CIPA) are shown. CPPs include force, gap and roll speed, whereas CIPAs preblend and ribbon porosity. Note: Actual rolls on the Mini-Pactor are inclined.

20.3.4.3 Knowledge Space (DoE)

The platform knowledge space is constructed from a factorial design, and its resulting predictive equations describe the relationship between the CIPAs (y variables, responses) and the formulation/process parameters (x variables, factors). The design approach applied to RC is illustrated in Figure 20.11. A CCF design was selected, as it allows for the quantification of squared terms and thus modeling of nonlinear behavior. Based on the initial risk ranking of process parameters, roll pressure and gap size were systematically varied along with the intra-granular content of APIs. As the RC process parameters are adjusted in real time, such a design can be completed from just a few API batches (one batch per API load). In the case of RC, the API particle size was kept fixed at a d_{50} of 65 μm. Preliminary tablet compaction trials confirmed that variations in particle size from 40–165 μm have only minor effects on the porosity of the resulting compact. As shown in Figure 20.11, API particle size is instead assessed during tablet compression against tablet CU (CQA). It is also noted that knowledge spaces for each unit operation (RC and tablet compression) are constructed independently of each other. This allows for unambiguous interpretation of the DoE data, since several of the factors (x) and responses (y) are confounded across the two unit operations. For example, ribbon porosity is a CIPA response variable (y) at the RC stage, while during tablet compression it may be regarded as a controllable input variable (x),

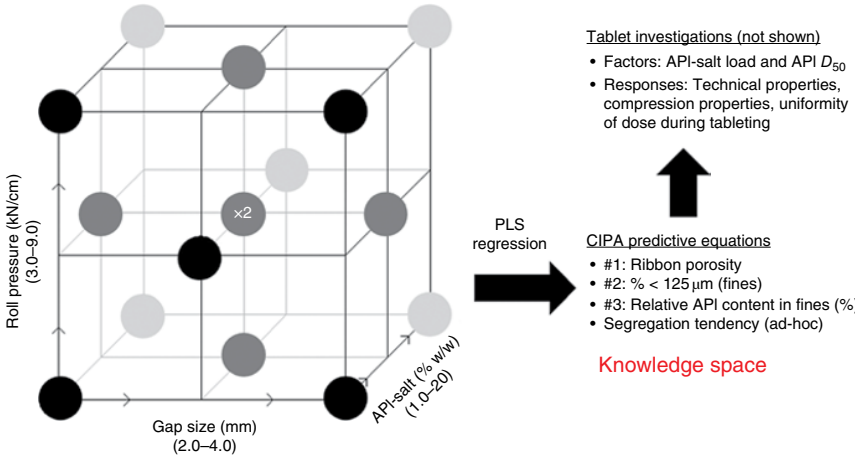


Figure 20.11 Illustration of the approach taken to develop the MCC-based RC platform formulation. The granulation knowledge space is constructed through a CCF optimization design. The derived CIPA predictive equations are used to calculate optimal process settings providing granules of acceptable quality. The resulting granulate is compressed into tablets from which the tablet knowledge space is independently generated.

affecting the manufacturability (y) of the tablet product, for example, hardness (see Figure 20.9). Merging the DoEs is therefore not meaningful and not recommended. Rather, a multivariate analysis using principal component analysis (PCA)/partial least squares (PLS) may later be conducted on the entire data sets (RC and tablet compression) in addition to the separate DoEs (data not shown) in order to investigate the mutual relationship between RC and tablet compression variables.

Once the DoE has been carried out, the coefficients (β) describing the relationship between CIPAs (y) and factors (x) are computed by suitable least squares regression term:

$$y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \text{squared terms} + \text{interaction terms} + \text{constant} \tag{20.4}$$

In this work, PLS was applied (MODDE, Umetrics MKS), as a more reliable alternative to multiple linear regression (MLR), due to covariance between responses. The model for ribbon porosity (CIPA-1) explained 92% of the total variance of the data set (R2) and 83% according to cross-validation (Q2). CIPA-2 (% fines < 125 µm) and CIPA-3 (relative API content in fines) provided inferior, yet acceptable, model performance with R2/Q2 of 75%/65% and 80%/71%, respectively (Table 20.4).

These predictive equations form the backbone of the platform formulation approach and may now be used to predict knowledge spaces for new APIs and at other intra-granular potency levels.

20.3.4.4 Application to Test Substance

A new Lundbeck molecule, suitable for processing by RC, was selected as test compound for verification of the platform applicability. As a first step, it was determined how well the properties of this new API matched the intended formulation strategy, as well as the ranges of formulation and process parameters variables (factor levels) covered by the platform. Table 20.5 shows selected properties of the platform and test formulation.

The API was compatible with the preferred filler, that is, MCC. However, the high dose necessitated a 28% API loading of the granulate, which exceeded the 1–20% w/w range investigated in the platform. The higher amount of API may cause problems such as poor powder flow (Risk #1), and thus poor feeding into the compaction zone, and insufficient compactibility of the powder blend for tableting (Risk #2) [12]. Risk #1 was mitigated by adding a small amount of glidant to the formulation. Risk #2 was not expected to cause issues, since preliminary compression studies on the test compound confirmed it had appropriate plastic behavior and a compressibility superior to the model substance used during the platform development. For that reason—and to further assure conformance with CU (CQA)—the target ribbon porosity (CIPA-1) for the 28% active granulate was set lower than usual, at 20–25%, which is known to

Table 20.4 Platform data output and PLS regression model performance.

		PLS model performance	
CIPA #	**Design data span (%)**	**R2**	**Q2**
1. Ribbon porosity	20–45	0.92	0.83
2. % < 125 µm (fines)	15–39	0.75	0.65
3. Relative API content in fines	20–66	0.80	0.71

Table 20.5 Platform and test compound property comparison.

Property	**Platform (PF) range**	**Test compound (TC)**	**TC within PF specs? (yes/no)**
Excipient selection	MCC/Mg-St	MCC/Mg-st	Yes (no stability concerns)
API load	1–20% w/w	9% (10 mg dose)	Yes
		28% (90 mg dose)	No (extrapolation)
API volume median particle size	d_{50}: 40–165 µm	~50–150 µm	Yes
API particle morphology	Brick-like	Platelike	No (may impact pre-blending and feeding)

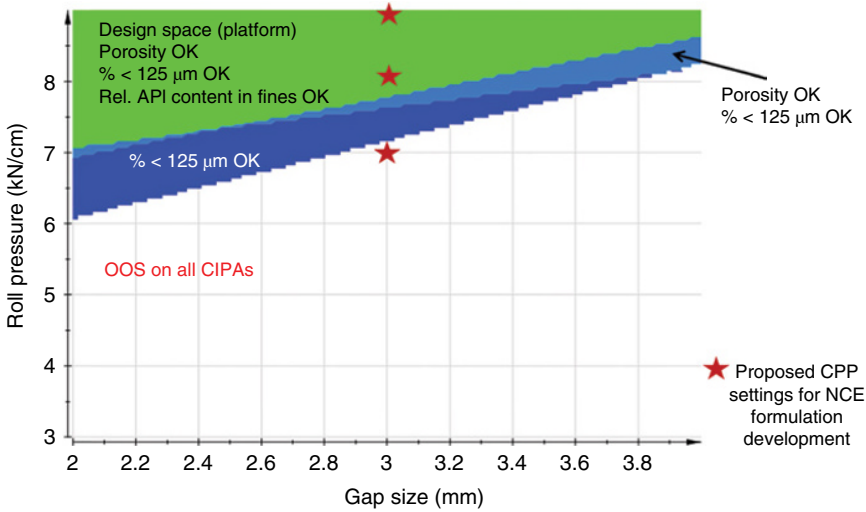


Figure 20.12 Design space regions for the MCC-based platform formulation at a 28% w/w API intra-granular load. Stars indicate the process parameter settings applied to the test compound. The design space for the 9% w/w granulate is not shown. OOS, out of specification.

lead to fewer fine granule particles and thus reduce the risk of segregation. As there was a need for a lower strength table in some markets, a 9% active granule was also developed, which was within the platform API load range and permitted a porosity range of 25–35%. The fines content, percentage < 125 µm (CIPA-2), should not exceed 30% and the relative API content in that fraction must be lower than 45% (CIPA-3). Using the predictive equations from the platform (model compound), the design space was calculated at the 9 and 28% intra-granular loadings. The extrapolated design space for the 28% active granulate is presented as a "sweet-spot" plot in Figure 20.12, showing which combinations of process parameters, roll pressure, and gap sizes are expected to provide a granule product of acceptable quality (upper region).

It was decided to manufacture at a gap size of 3.0 mm and the three starting points for roll pressure were selected. The first was within the upper region (9.0 kN/cm), where all CIPAs are within specifications; the second at the edge of failure (8.0 kN/cm); and the last one at 7.0 kN/cm, where all CIPAs are out of specification. These constitute the very first experiments to be carried out at small scale. Approximately 500 g of pre-blend for each roll pressure was roller compacted and compressed into tablets on a single punch press. The measured CIPA results from the initial screening (Campaign I) are listed in Table 20.6 along with larger scales (Campaigns II and IV) and one batch where the API specifications was tested and where other API qualities were used (Campaign III).

Table 20.6 Results from roller compaction of the test formulation including CIPA predictions from the platform.

				CIPA-1		CIPA-2		CIPA-3	
				Ribbon porosity (%)		% < 125 µm		Rel. API content in fines (<125 µm)	
Batch campaign	API batch #	Potency (% w/w)	Roll pressure (kN/cm)	Measured ($n = 3$)	Predicted (95% conf.)	Measured	Predicted	Measured	Predicted
I (500 g scale)	A	28%	7.0	24 ± 1%	27 ± 3%	N/A	N/A	N/A	N/A
		28%	8.0	22 ± 0%	24 ± 3%	N/A	N/A	N/A	N/A
		28%	9.0	18 ± 1%	20 ± 3%	N/A	N/A	N/A	N/A
II (10 kg scale-up)	B	9%	6.5	30 ± 1%	32 ± 1%	45%	26 ± 1%	56%	43 ± 3%
		28%	9.0	20 ± 1%	20 ± 3%	32%	27 ± 4%	32%	40 ± 7%
III (API morphology variations)	C	28%	8.0	20 ± 0%	24 ± 3%	35%	29 ± 3%	N/A	N/A
	D	28%	8.0	19 ± 0%	24 ± 3%	34%	29 ± 3%	N/A	N/A
	E	28%	8.0	20 ± 0%	24 ± 3%	33%	29 ± 3%	N/A	N/A
IV (30 kg scale-up)	F	9%	6.5	31 ± 1%	32 ± 1%	33%	26 ± 1%	24%	43 ± 3%
		28%	8.0	22 ± 1%	24 ± 3%	38%	29 ± 3%	28%	44 ± 7%

The measured ribbon porosities in Campaign I were in close agreement with predicted values from the platform. The applicability of the platform with respect to ribbon porosity was also confirmed at the 10 and 30 kg scale (Campaigns II and IV), albeit discrepancies between predicted and measured values of CIPA-2 and CIPA-3 were found. The 10 kg batch (Campaign II) had a markedly higher fines content (CIPA-2) and fines potency (CIPA-3) than predicted by the platform model. The bimodal distribution of RC granules makes it difficult to perform representative sampling. The platform formulation was prepared on a small scale, and granules were collected in real time during granulation (500 g per sub-batch in the DoE). Sampling is therefore expected to be representative for the platform formulation. In contrast, granules from the 10 kg batch with the test compound were collected after manufacturing of the entire 10 kg batch. Hence, the collected granules may contain more fines than the average content of the entire 10 kg batch. Granules from Campaigns III and IV were collected in real time. Finally, the inaccuracy of the PLS models of CIPA-2 and CIPA-3 also adds to the overall uncertainty (see previous section).

Taking into account the higher compressibility (lower ribbon porosity) of the new API (Campaign III), it was decided to manufacture future 28% w/w batches at an 8.0 kN/cm roll pressure and to utilize 6.5 kN/cm for any future 9% w/w batches. For the 28% w/w loading, this roll pressure provides a ribbon porosity between 19 and 22%, irrespective of the API batch used, which is within the intended 20–25% originally targeted with the platform model. Subsequent tablet compression (results not shown) demonstrated excellent tablet compactibility (20–110 N) even for granules processed from low porosity ribbons (9.0 kN/cm).

With respect to establishment of a knowledge space on tablet manufacturability, it was shown in a previous section that this should be constructed separately from the intermediate granule product in order to preserve a high degree of interpretability. In RC, ribbon porosity—a CIPA response variable in the RC process (see Figure 20.9)—becomes a controllable input parameter in the tablet compression process. It is therefore possible to carry out a separate DoE quantifying the effect of tablet punch force and (ribbon) porosity of the granules on tablet hardness. Response surfaces of such correlations for the test substance are shown in Figure 20.13. It is possible to gain control over tablet hardness via applied punch force for the relevant ranges of ribbon porosity (see Table 20.6).

This example demonstrates how existing formulation and process knowledge of a model compound can be accumulated in a platform technology and applied to a new test compound. This allows the development scientist to quickly make risk- and science-based decisions on formulation composition and process parameters without sacrificing process understanding and control

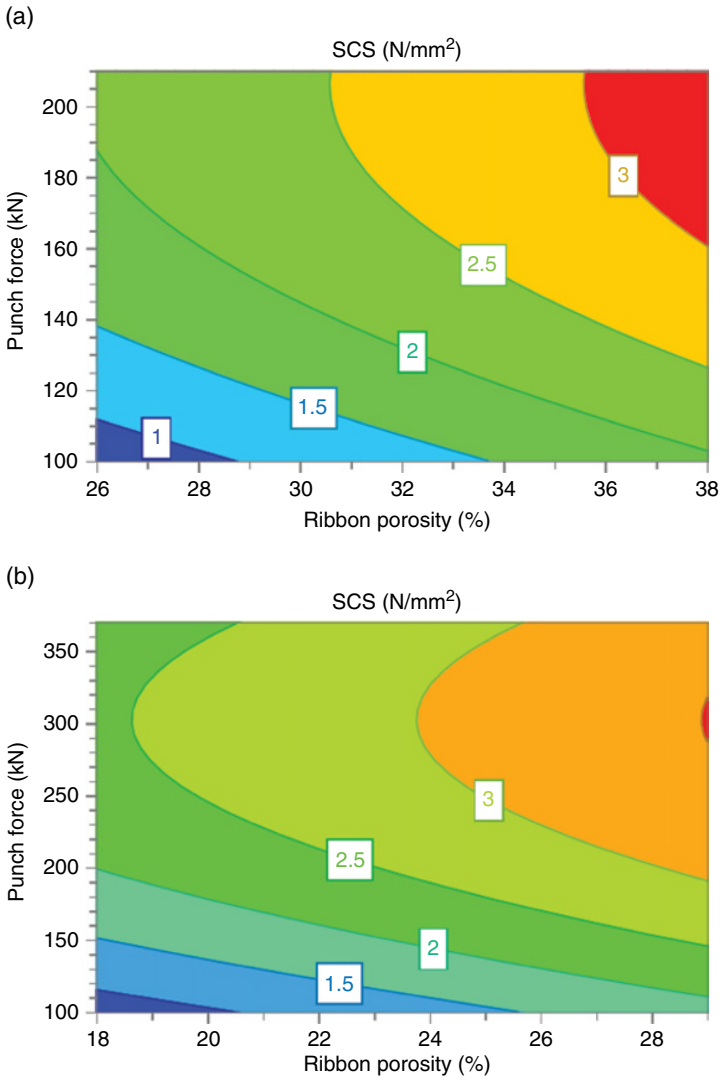


Figure 20.13 Response surface plots of specific crushing strength (SCS) (i.e., hardness normalized against tablet cross sectional area) for the (a) 9% granulate and (b) 28% using the test substance.

of product quality. In the case of RC, it took only one working day on the compactor to verify the feasibility of the formulation choice and process settings suggested by the platform strategy. The approach taken to construct and apply the RC platform is summarized in Figure 20.14.

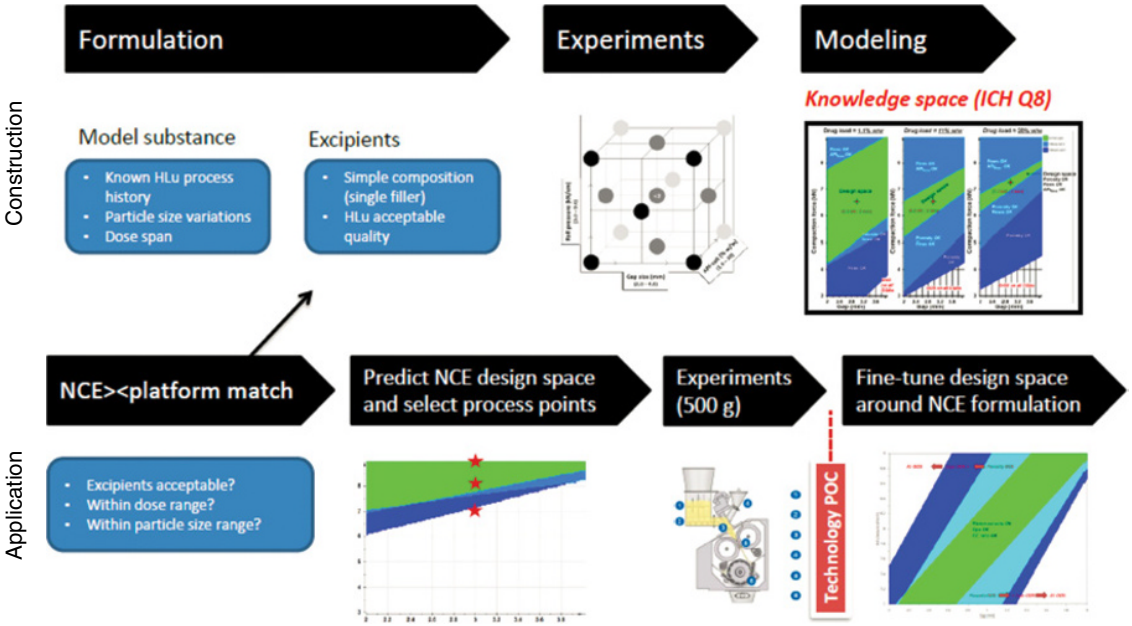


Figure 20.14 Summary of the construction and application of the roller compaction platform. POC, proof of concept. At this early stage of NCE formulation development it is known whether the API can be processed by roller compaction at a larger scale.

20.3.4.5 Understanding Scale-Up

The aim of a successful scale-up exercise is to create similar processing conditions for the product across different scales via the appropriate adjustment of process parameters. An appropriate scaling factor may be either found through empirical testing at the larger scale or derived more rationally from FPM. However, scale-up from lab/pilot to commercial scale can pose challenges that are difficult to foresee as well as mitigate, not least because these issues occur late in development where the formulation composition is often fixed. As such permissible scale-up control strategies are confined to the adjustment of process parameters as well as design alterations, the latter typically only realistic with new equipment as a part of its installation and qualification program. Proper understanding of scale-up parameters stems from mechanistic insight into the given process technology and its various scales. It is therefore rational to investigate these as a part of the technology platform and apply the knowledge proactively to future products. Examples of FPM approaches are thermodynamic mass–energy balance calculations and nozzle droplet size distribution in FBWG and calculations of the nip angle/area in dry granulation when switching to larger roll diameters [45]. In RC, the nip angle indicates the start of powder densification and therefore determines the total pressure applied to the powder at a given roll force and ultimately impacts ribbon porosity. Assuming a fixed granulator sieve size between the two scales, ribbon porosity is the most important quality descriptor of a RC granule's compression properties and size distribution. This is evident in Figure 20.15 showing the relationship between ribbon porosity and the granule geometric mean diameter (D_{gw}) for sub-batches of both API loadings of 9 and 28% (described in previous sections). The powder blends have been roller compacted at various combinations of roll pressure and gap size to produce ribbon porosities covering 26–38 and 18–29% for the 9 and 28% loads, respectively. For both blends there is a linear relationship between ribbon porosity and resulting granule size irrespective of API loading, that is, the harder the ribbons, the fewer the number of fine particles, and vice versa.

Therefore, when scaling up a roller compactor process, one should focus on ribbon porosity as the main scale-up attribute. As an alternative to FPM, Liu et al. [46] modeled historic data from a small-scale RC by Joint-Y PLS regression and applied this to predict the scale factor for roll pressure on a larger scale compactor with a larger roll diameter. However, some of the scale-up challenges in RC may also be averted through innovative design of the different equipment scales. The flagship models of Gerteis compactors include the Mini-Pactor and the Macro-Pactor. A scale-comparable front view of the two compactors is shown in Figure 20.16. The Mini-Pactor is often referred to as *pilot scale* although the continuous nature of this unit operation makes it suitable for both lab scale and, in principle, production

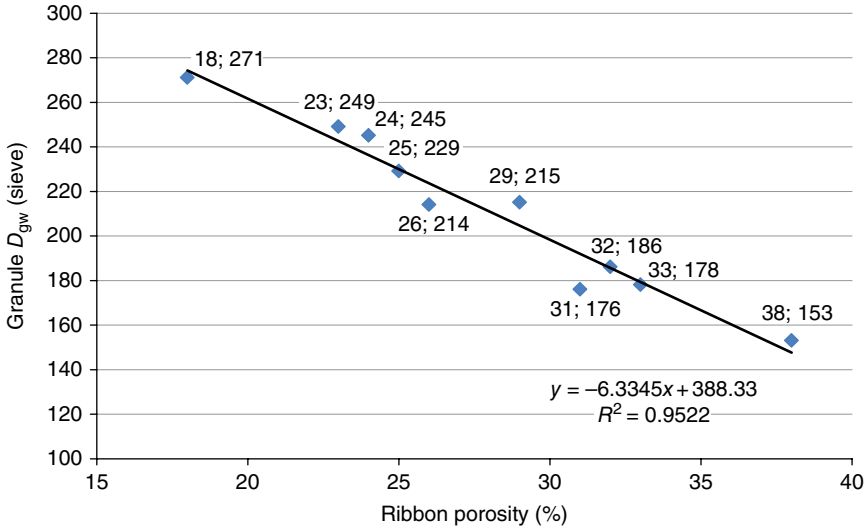


Figure 20.15 Relationship between ribbon porosity and granule geometric mean size (µm) of five batches each of the 9 and 28% w/w (API) blends described in previous sections. All granulates were subdivided prior to sieve analysis to ensure representative sampling and the most accurate determination of D_{gw}.

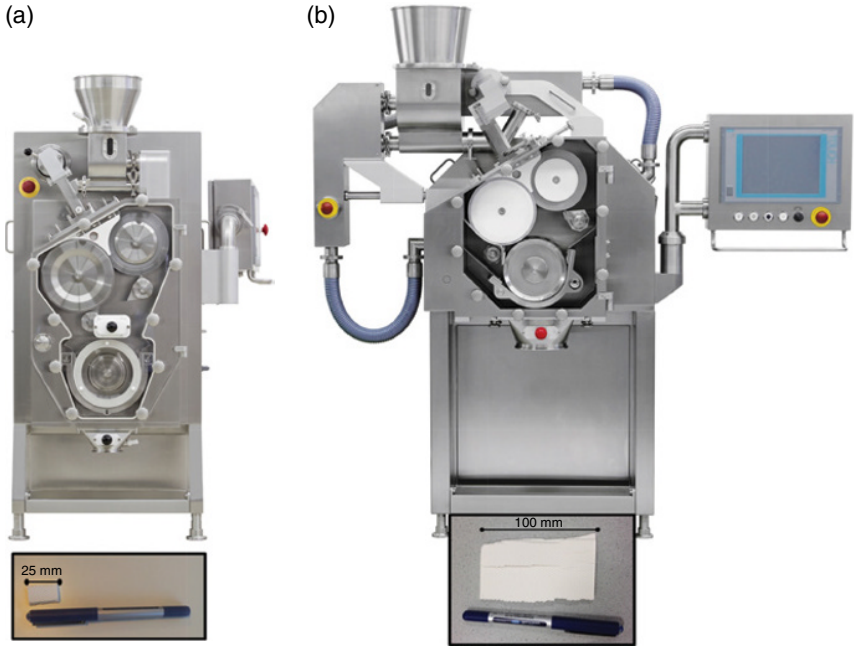


Figure 20.16 Scale-comparable front view of Gerteis' (a) Mini-Pactor and (b) Macro-Pactor roller compactors. The rolls—and thus the ribbons—are four times wider for the Macro-Pactor as shown in the bottom of the picture.

scale depending on the processing time. The Macro-Pactor is Gerteis' *production scale* compactor. It is very similar to the Mini-Pactor, the two most notable differences being the wider press rollers (25 vs. 100 mm) and a twin auger feeding system (vs. single feed on Mini-Pactor) to accommodate for the larger throughput. The rolls have identical diameters between the two scales, which ensure an identical nip area and thus a similar impact of roll pressure and gap size on blend. Consequently, ribbon porosity is claimed to be scale independent. To the best of our knowledge no studies exist that can corroborate this hypothesis, and it is therefore rational to investigate this claim as a part of the platform.

Two MCC-based placebo mixtures were used in these investigations and formulated to obtain different flow properties, thereby challenging the feeding system on the larger Macro-Pactor. A mixture composed of solely Avicel PH101 and lubricant represents the poorest flowing placebo batch and was categorized as borderline cohesive/easy-flowing according to shear testing investigations. The second placebo batch contains the better flowing Avicel PH102, as well as lubricant and glidant, and was categorized as borderline easy flowing/freely flowing. To confirm the claimed 1 : 1 scalability of roll pressure and gap, each test mixture was processed on both compactor scales using identical RC parameter settings. Roll force and gap were varied systematically according to a $2^2 + 1$ DOE screening design. Levels for gap were 2.0–4.0 mm, while the ranges for applied force were 5.0–9.0 kN/cm. These combinations of ranges are known from experience to provide ribbon porosities of between 20 and 40%, which is a realistic range (as described in earlier sections).

Ribbon porosity results of the Avicel PH101 and PH102 placebo batches, as well as values predicted using the platform described earlier, are shown graphically in Figure 20.17. Since the RC platform for MCC-based formulations were developed using API loadings in the range 1–20% w/w (see previous section), an API loading of 1% w/w is used to approximate the placebo mixtures in this project and thus avoid extrapolation. The equation is expected to work also for Avicel PH101 due to similar particle densities of the PH101 and PH102 grades. Generally, there is good agreement between the ribbon porosities of the equipment on the two scales, with a deviation from 0 to a maximum of 3% between the two scales.

The results confirm the expected 1 : 1 scalability of the Mini-Pactor to the Macro-Pactor and demonstrate how a logical equipment design can mitigate potential scale-up risks. This markedly reduces the time spent by the formulator on scale-up investigations and thereby allows them to focus on other scale-up risks like punch adhesion and segregation of the roller compacted granulate prior to tablet compression. These risks are heavily API dependent and are therefore most rationally investigated during NCE formulation development.

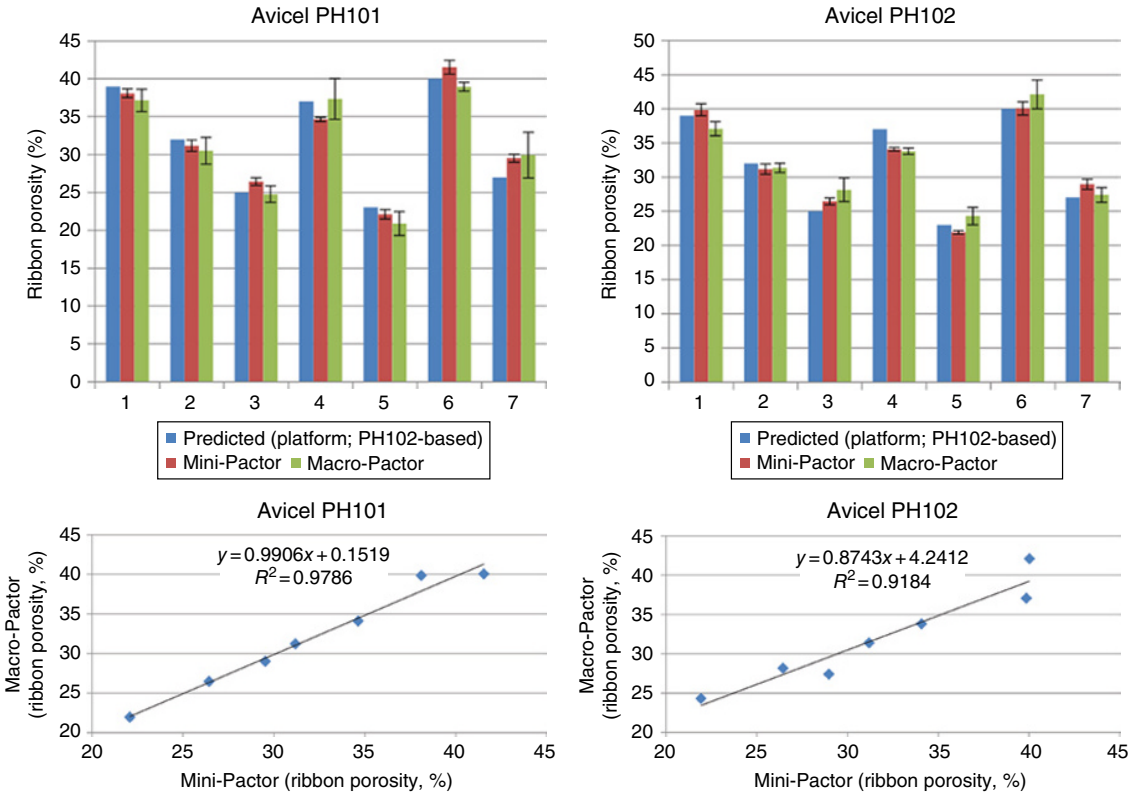


Figure 20.17 Column charts (top) and scatter plots (bottom), the latter confirming a 1 : 1 relationship between the porosity of ribbons acquired from the Mini- and Macro-Pactor at identical gap and roll force.

20.4 Conclusion

In general, ICH Q8(R2) [11] encourages pharmaceutical companies to build quality into their products by combining sound science-based development work with QbD using risk management and DoE for evaluating critical formulation and process parameters that subsequently impact CQAs. The most important benefit is that this systematic and rational development of a product using QbD principles ensures a robust and reliable quality of the final product with less risk of failure during its life cycle and consequently a reduced economical risk to the manufacturer and improved safety for the patient. Further, this helps to establish a design space within which parameters may be adjusted to deliver the intended QTPP. It must, however, be emphasized that ICH Q8(R2) [11] does not exclude the application of an empirical based development approach, which might be more cost-effective in the case of simple or small volume products.

The key tools in rational product development of a solid dosage form containing a NCE, according to QbD, might include the following elements:

- Rational selection criteria for choice of excipients [29] and processing based on public and prior knowledge embedded into internal decision tools applying risk management approaches and DoE
- Application of FPM (if possible) and mechanistic knowledge supporting choice of process settings, formulation specifications, for example, using models to explore the effect of particle size of drug on CU or dissolution, and subsequent control strategies
- Verification of the applicability of formulation technology platforms to the specific NCE and eventually adjustment of the design space accordingly

It is our experience that this approach results in a significantly reduced number of product and process related variables that need to be investigated during product development. Further, as prior knowledge is gained throughout the development of a number of products, the generic tools and platforms technologies can be continuously improved and expanded. This includes updating information contained in databases/tables and/or adding more data to the predictive equations from the initial DoE. These measures ensure a flexible, yet strong platform base within the organization. In order to realize the full benefits of a platform technology, it is important to consolidate the results of the platform work into user-friendly tools, for example, as an internal development handbook, and to train both academics and technicians working within product development. Risk assessment [12] is an integral part of all NCE formulation development, and documentation is essential to support statements of criticality. The knowledge generated in the platform may be

deemed *prior knowledge* and is added to regulatory templates for a given formulation and the associated process technology. Hence, parts of the regulatory file can be prepared in advance, at the early stages of NCE formulation development, and with full documentation from the platform to support the risk ranking approach.

In summary, the technology platform plays a key role in our work and supports a QbD-based formulation development, and the concept should be considered for all pharmaceutical drug delivery systems.

References

1 EU. 2014. Pharmaceutical Quality System. In: *Guidelines for good manufacturing practice for medicinal products for human and veterinary use*. https://ec.europa.eu/health/documents/eudralex/vol-4_en. Accessed on July 6, 2017.

2 Rathore AS, Mhatre R. (Eds). 2011. *Quality by design for biopharmaceuticals: principles and case studies*. Hoboken: John Wiley & Sons, Inc.; 2011.

3 Elliott P, Billingham S, Bi J, Zhang H. 2013. Quality by design for biopharmaceuticals: a historical review and guide for implementation. *Pharmaceutical Bioprocessing* 1; 105–122.

4 Yu LX, Amidon G, Khan MA, Hoag SW, et al. 2014. Understanding pharmaceutical quality by design. *The AAPS Journal* 16; 771–783.

5 Juran JM. 1992. *Juran on quality by design: the new steps for planning quality into goods and services*. New York: The Free Press; 1992.

6 FDA's Critical Path Initiative. 2004. http://www.fda.gov/ScienceResearch/SpecialTopics/CriticalPathInitiative/ucm076689.htm. Accessed on June 4, 2016.

7 Food and Drug Administration CDER. 2004. Guidance for industry, PAT: a framework for innovative pharmaceutical development, manufacturing, and quality assurance. September, 2004.

8 Food and Drug Administration. 2004. Pharmaceutical CGMPs for the 21st century: a risk based approach. September, 2004.

9 Food and Drug Administration. 2005. Submission of chemistry, manufacturing and controls information in a new drug application under the new pharmaceutical quality assessment system; notice of pilot program. *Federal Register* 70; 40719–40720.

10 Van Buskirk GA, Asotra S, Balducci C, Basu P, et al. 2014. Best practices for the development, scale-up, and post-approval change control of IR and MR dosage forms in the current quality-by-design paradigm. *AAPS PharmSciTech* 15; 665–693.

11 ICH Q8(R2). 2009. Pharmaceutical development. Current step 4 version, August, 2009.

12 ICH Q9. 2005. Quality risk management. Current step 4 version, November 9, 2005.
13 ICH Q10. 2008. Pharmaceutical quality system. Current step 4 version, June 4, 2008.
14 ICH Q11. 2012. Development and manufacture of drug substances (chemical entities and biotechnological/biological entities). Current step 4 version, May 1, 2012.
15 Kushner J, Langdon BA, Hicks I, Song D, et al. 2014. A quality-by-design study for an immediate-release tablet platform: examining the relative impact of active pharmaceutical ingredient properties, processing methods, and excipient variability on drug product quality attributes. *Journal of Pharmaceutical Sciences* 103; 527–538.
16 Huang J, Kaul G, Cai C, Chatlapalli R, et al. 2009. Quality by design case study: an integrated multivariate approach to drug product and process development. *International Journal of Pharmaceutics* 382; 23–32.
17 Peterson JJ, Lief K. 2010. The ICH Q8 definition of design space: a comparison of the overlapping means and the Bayesian predictive approaches. *Statistical Biopharmaceutical Research* 2; 249–259.
18 The Common Technical Document. 2002. The common technical document for the registration of pharmaceuticals for human use: Quality—M4Q(R1) Quality overall summary of module 2, module 3: Quality. Current Step 4 version, September 12, 2002.
19 Yu LX. 2008. Pharmaceutical quality by design: product and process development, understanding, and control. *Pharmaceutical Research* 4; 781–791.
20 ICH Q6A. 1999. Specifications: test procedures and acceptance criteria for new drug substances and new drug products: chemical substances. Current step 4 version, October 6, 1999.
21 ICH. 2012. Guidance for industry Q8, Q9 and Q10, questions and answers including appendix for training sessions.
22 Serajuddin ATM, Thakur AB, Ghoshal RN, Fakes MG, et al. 1999. Selection of solid dosage form composition through drug-excipient compatibility testing. *Journal of Pharmaceutical Sciences* 88; 696–704.
23 Verma RK, Garg S. 2005. Selection of excipients for extended release formulations of glipizde through drug-excipient compatibility testing, *Journal of Pharmaceutical and Biopharmaceutical Analysis* 38; 633–644.
24 ICH Q1A(R2). 2003. Stability testing of new drug substances and products. Current step 4 version, February 6, 2005.
25 Food and Drug Administration CDRH. 2011. Draft guidance for industry and food and drug administration staff: applying human factors and usability engineering to optimize medical device design. June, 2011.
26 ISO Standard No. 62366. 2007. Medical devices: application of usability engineering to medical devices.

27 Awotwe-Otoo D, Agarabi C, Wu GK, Casey E, et al. 2012. Quality by design: impact of formulation variables and their interactions on quality attributes of a lyophilized monoclonal antibody. *International Journal of Pharmaceutics* 438; 167–175.

28 Nazzal S, Nutan M, Palamakula A, Shah R, et al. 2002. Optimization of a self-nanoemulsified tablet dosage form ubiquinone using response surface methodology: effect of formulation ingredients. *International Journal of Pharmaceutics* 240; 103–114.

29 Elder DP, Kuentz M, Holm R. 2016. Pharmaceutical excipients: quality, regulatory and biopharmaceutical considerations. *European Journal of Pharmaceutical Sciences* 87; 88–99.

30 Food and Drug Administration. 2011. Process validation: general principles and practices. Guidance for industry. January 2011.

31 ISPE Guide Series. 2011. Product Quality Lifecycle Implementation (PQLI®) from concept to continual improvement part 1: Product realization using QbD, concepts and principles.

32 ISPE Guide Series. 2011. Product Quality Lifecycle Implementation (PQLI®) from concept to continual improvement part 2: Product realization using quality by design (QbD): illustrative example. http://wwww.ispe.org/pqli-guides/product-realization-qbd-example. Accessed on April 8, 2017.

33 Allesø M, Torstenson AS, Bryder M, Holm P. 2013. Presenting a rational approach to QbD-based pharmaceutical development: a roller compaction case study. *European Pharmaceutical Review* 6; 17–24

34 Halman JIM, Hofer AP, van Vuuren W. 2003. Platform-driven development of product families: linking theory with practice. *Journal of Product Innovation Management* 20; 149–162.

35 Fiil-Nielsen O, Mortensen NH. Implementing product platforms: a case study. International Design Conference: Design 2006, Dubrovnik, Croatia, May 15–18, 2006.

36 FDA, 2016. Biopharmaceutics classification system (BCS) guidance. http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm128219.htm. Accessed on June 10, 2016.

37 Leane M, Pitt K, Reynolds G. 2014. The Manufacturing Classification System (MCS) Working Group. A proposal for a drug product Manufacturing Classification System (MCS) for oral solid dosage forms. *Pharmaceutical Development Technology* 20(1); 1–10.

38 Baertschi SW, Alsante KM, Santafianos D. 2011. Stress testing: the chemistry of drug degradation. In: Baertschi SW, Alsante KM, Reed RA. (Eds). *Pharmaceutical stress testing: predicting drug degradation*. London: Inform Healthcare, pp. 49–141.

39 Johnson MCR. 1972. Particle size distribution of the active ingredient for solid dosage forms of low dosage. *Pharmaceutica Acta Helvetica* 47; 546–553.

40 Hintz RJ, Johnson KC. 1989. The effect of particle size distribution on dissolution rate and oral absorption. *International Journal of Pharmaceutics* 51; 9–17.

41 Noyes AA, Whitney WR. 1897. The rate of solution of solid substances in their own solutions. *Journal of American Chemical Society* 19; 930–934.

42 Kleinebudde P. 2004. Roll compaction/dry granulation: pharmaceutical applications. *European Journal of Pharmacy and Biopharmaceutics* 58; 317–326.

43 Yue T, Qui Z, Wen H. 2009. Systematical approach of formulation and process development using roller compaction. *European Journal of Pharmacy and Biopharmaceutics* 73; 219–229.

44 Soh JLP, Wang F, Boersen N, Pinal R, et al. 2008. Utility of multivariate analysis in modeling the effects of raw material properties and operating parameters on granule and ribbon properties prepared in roller compaction. *Drug Development and Industrial Pharmacy* 34; 1022–1035.

45 Reynolds G, Ingale R, Roberts R, Kothan S, et al. 2010. Practical application of roller compaction process modeling. *Computational Chemical Engineering* 34; 1049–1057.

46 Liu Z, Bruwer M-J, MacGregor JF, Rathore SSS, et al. 2011. Scale-up of a pharmaceutical roller compaction process using a joint-Y partial least squares model. *Industrial Engineering and Chemical Research* 50; 10696–10706.

21

ICH Q9 Quality Risk Management

David Elder[1] *and Andrew Teasdale*[2]

[1] *David P Elder Consultancy, Hertford, UK*
[2] *AstraZeneca, Macclesfield, UK*

21.1 Introduction

ICH Q9 [1] together with ICH Q8(R2) [2], Q10 [3], and Q11 [4] is designed to facilitate the development of science-centered and risk-based approaches to pharmaceutical quality. ICH Q9 [1] itself is intended to focus on the behaviors of industry and the regulatory authorities on the two primary principles of quality risk management (QRM), which are as follows:

- The evaluation of the risk to pharmaceutical quality should be based on scientific knowledge, directly linked to patient safety; and the level of effort, formality, and documentation of the QRM process should be commensurate with the level of risk.
- An effective QRM process provides a key mechanism for the proactive identification and subsequent control of potential issues that may arise during development and subsequent commercialization of a drug product. QRM can be delineated as shown in Table 21.1.

21.1.1 Quality: Definition

So what is quality? This is not an easy question to answer. Several commentators have reflected that quality per se is difficult to define, that is, "definitions are a product of rigid formal thinking, thus quality cannot be defined" [5]. However one cannot ignore the need to define quality however intangible it may seem. Put simply, quality needs to be adequately defined to meet the

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Table 21.1 Quality risk management definitions.

Parameter	Definition
Quality	The degree to which a set of intrinsic properties of a drug substance or drug product, in conjunction with its underpinning manufacturing process, and any supporting processes, which when combined fulfill the predetermined criteria required to ensure its intended use
Risk	The combination of the probability of occurrence of harm (or unwanted outcome) and the severity of that harm
Management	The establishment of systematic procedures for the assessment, control, communication (both within and out with the organization), and iterative review of risks to the quality of the drug substance or drug product across the product's life cycle (across development and subsequent manufacture)

future needs of the customer [6]. However it must be recognized that quality has multifactorial attributes ensuring that quality can never be defined in terms of a single attribute [7, 8].

The American Society for Quality (ASQ) argues that "quality can have two meanings: a product or service free of deficiencies and the characteristics of a product or service that bear on its ability to satisfy stated or implied needs" [9]. Hoyer and Hoyer [10] agreed, indicating that quality involves both conformance to specification (level 1) and customer satisfaction (level 2) attributes. Some commentators focus primarily on the former or latter definitions, whereas others reflect on both facets. Some define quality from both perspectives, as fitness for use and freedom from deficiencies. Interestingly, quality is defined by ICH Q6A [11] as fitness for use, while ICH Q9 [1] defines quality as conformance to requirements [6]. Montgomery [7] raised the important point that process capability and specification limits were integrally linked and that "quality is inversely proportional to variability." Additionally, quality cannot be addressed by solving manufacturing issues as and when they occur [7]; quality must be designed into the product [2]. The tenant of designing in quality is of course central to the philosophy of ICH Q8 [2] and Q11 [4].

However, with the globalization of medicinal supply chains, it is important to address the issue of quality from a global perspective. Indeed, the availability of poor quality medicines (including adulterated and counterfeit products) is a significant public health issue. Concerns include subpotent, supra-potent, absent, or indeed different active ingredients, contamination, poor quality ingredients, poor quality manufacturing sites, inadequate stability, and inappropriate packaging [12]. Unfortunately, there are multiple causes often

prompted by deliberate intent, that is, counterfeiting or adulteration. In some regions, drugs produced for export may not be regulated to the same standards as those intended for internal use, this is compounded in some countries by the fact that the regulatory agencies are inadequately equipped to monitor and control the problem [12]. Unfortunately, counterfeiting is not restricted to API or drug products; there is a growing trend to adulterate common excipients with cheaper ingredients [13]. Worryingly, the best documented examples typically result in fatalities. For example, contamination of glycerine with diethylene glycol resulted in over 200 deaths in Panama City [14] and has belatedly led to the introduction of tests for diethylene glycol and ethylene glycol as potential adulterants in several pharmacopoeia [13]. It may be concluded from this that quality is best defined from the consumer's perspective as fitness for purpose, that is, the product is suitable for the intended purpose [15].

Although in most other fields of commerce the voice of the customer is paramount with drug products, the customer is rarely able to discern the quality of the product, with the obvious exception of customer complaints [16]. As a case in point, consider the willingness of customers to buy prescription drugs from the Internet, despite limited (if any) assurances of product quality [17]. Indeed, patients typically seek effective, safe, and affordable drugs, and all other considerations, for example, appearance, taste, and smell, may be secondary. The economic cost of quality is therefore not determined by consumer demand, but by governmental oversight. Thus, risk affords a more suitable basis for the measurement of drug product quality rather than economic factors [18]. Any effective QRM thus needs to consider the product from a holistic perspective focused on every aspect, including supply chain integrity as well as control of the manufacturing process itself.

21.1.2 Risk

Scientific tools together with experience and expertise are used to estimate the likelihood of any given risk [1], but it is the role of the regulators to articulate what is acceptable. An extreme, some would say excessive, approach to this "protective role" of the regulators is the concept of "zero risk." This approach was embraced by the US Congress during the re-drafting of the Food, Drug and Cosmetic (FD&C) Act [19]. Manufacturers were required by law to demonstrate the safety of food and color additives. The "Delaney Clause" of the FD&C act banned the use of any additives known to cause cancer in man, or experimentally, in animals. This was initially opposed by scientists and regulators alike, who argued that banning these additives in food at very low levels solely on the ability to cause cancer in animals at very high levels was not scientifically sound. However, this approach was politically expedient and FDA was

required by law to apply a "zero-risk tolerance" approach to food additives. By the early 1970s it became clear that this approach was untenable and would lead to a large-scale prohibition of animal feed products [20]. In parallel, there was a clearer understanding that exposure to small quantities of food additives does not significantly increase the chance of contracting cancer, that is, there is a risk/benefit ratio. This prompts the intriguing question, is zero risk attainable or even desirable?

Over the last 50 years, the UK governments' general approach toward the responsibility of the regulatory agencies toward risk management is to maintain risks "as low as reasonably practicable" (ALARP) or "as low as reasonably achievable" (ALARA). Thus there is a balance between the risks encountered versus the expended effort (cost, time, and effort). If the expended effort is balanced versus the likely benefits accruing from risk reduction, then the appropriate "duty of care" has been achieved and supplementary approaches are not required [20]. Risks are then classified as unacceptable, acceptable, or tolerable, and efforts should be initiated to either eliminate (where feasible or desirable) or reduce unacceptable risks to acceptable levels. Acceptable risks are controlled at very low levels. Tolerable risks are defined by regulators, groups, or company policy. This approach reflects an underlying philosophy that resolution of risk should be approached from a standpoint of "safe enough," rather than from an absolute perspective of safe or unsafe [21]. This in turn led to the tolerability of risk (ToR) proposition [20], where tolerable does not mean acceptable, but instead implies a "willingness by society as a whole to live with a risk so as to secure certain benefits in confidence that the risk is one that is worth taking and that it is being properly controlled." That is, in the specific context of pharmaceuticals, it is where an appropriate risk/benefit balance has been achieved and where the additive benefits to the patient outweigh any inherent risk.

In the development of oncology medicines, for example, investigative clinical studies typically involve patients whose disease state is progressive and often fatal. In addition, the dose(s) used in these clinical studies often approach the adverse effect level defined by pre-clinical studies. Therefore, the design of those safety studies supporting oncology medicines can significantly differ from studies performed for other pharmaceuticals. Thus, as the clinical outcomes are potentially extremely poor, the tolerable risk can be significantly higher (in theory) as any therapeutic benefit seen is clearly beneficial. An exemplar of this is found in the development of oncology products and relates to impurities. ICH S9 [22] indicates that it is appropriate to propose that mutagenic impurities can be controlled at higher levels than proposed in the guideline for mutagenic impurities (ICH M7 [25]); however, it doesn't state that the standard guidelines for ordinary impurities (ICH Q3A(R2) [23]/ Q3B(R2) [24] can necessarily be utilised – this latter approach would require regulatory justification.

Delaney [26] was in agreement with this more pragmatic approach, observing that

> Should the issue of genotoxic impurity limits in pharmaceuticals be resolved from the perspective of historical precedent (the zero risk tolerance mindset), or from a more carefully considered cancer-risk avoidance and risk management view point that weighs the new regulatory burdens created against the benefits conferred by pharmaceutical products to patients?

One of Delaney's [26] main propositions from a risk management perspective was that the ICH M7 [25] guidance is often blind to the reality that pharmaceutical syntheses require the use of highly reactive intermediates (DNA reactive, i.e., mutagenic) in order to manufacture complex drug substances with high molecular weight (MW). Delaney [26] estimated that about 25% of all reactive intermediates are mutagenic. By stressing the need to avoid mutagenic intermediates whenever possible, the guidance sets an unrealistic expectation for chemists to spend more time and effort embracing synthetic approaches that may be considerably less efficient, giving lower yields and ironically less pure API (reactive intermediates are easier to purge than nonreactive ones), without materially improving patient safety.

21.2 Quality Risk Management (QRM)

21.2.1 Introduction

QRM has the overarching objective of protecting the patient from harm by minimizing the risk(s) to quality of the drug product. Patient risk cannot be completely avoided, but risk reduction strategies should be enacted to minimize both the probability and the severity of that risk to an acceptable level.

QRM involves a methodical approach toward the assessment, control, communication, and subsequent review of risks to the quality of the API and drug product across its entire life cycle. Although ICH Q9 [1] specifically focuses on drug product, it is clear that a full understanding of the API is pivotal to that goal, as the API is often one of the most significant contributors to the variability of all of the input parameters. A prototypical model for QRM is shown in Figure 21.1. However, ICH Q9 [1] makes clear that other suitably justified models could be used if appropriate. The specific focus on each component within the structure might vary from case to case (and site to site), but a robust QRM process will include assessment(s) of all the of the univariate and multivariate parameters at an appropriate level of detail that is suitable for each specific risk.

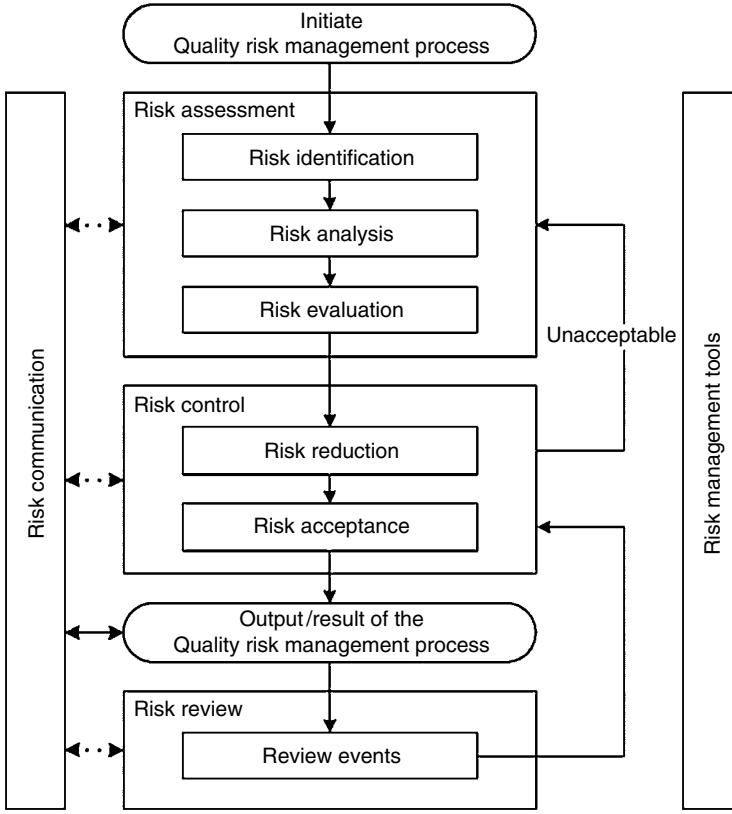


Figure 21.1 Outline of a typical QRM process. *Source:* ICH Q9 [1]. Reproduced with permission of ICH Q9.

NOTE: Decision points are not presented in Figure 21.1 as these decisions can occur at any part of the process. Indeed, these decision points might conclude the need to reassess the previous stage and seek further data or to modify the risk model or even to stop the QRM process based upon supporting information.

NOTE: "Unacceptable" in the process flow does not only infer any "statutory, legislative, or regulatory requirements," but also the requirement to reconsider the QRM process.

QRM assessments are usually undertaken by multidisciplinary teams containing subject matter experts (SMEs) from the appropriate business areas, for example, production, engineering, quality assurance, quality control (QC), regulatory affairs, statistics and R&D (Research & Development) (including clinical, if required), and commercial business. QRM teams often employ

facilitators (or enablers) who are knowledgeable about QRM processes. Accountability is a key aspect of any quality management system, thus an accountable person(s) (i.e., QRM facilitator(s)) who is responsible for directing and coordinating the QRM process, including ensuring that adequate resources are available, is an integral part of the team.

21.2.2 Implementing a QRM Process

It is important to recognize that QRM is an iterative process(es). This typically involves the following sub-process:

1) Definition of the problem statement including any assumptions governing the risk potential, typically phrased in the following way, "Because of x there is a risk that Y might happen…"
2) Collation of the available information and/or data pertaining to the possible hazard, potential harm, and/or likely impact on the patient. This information may be accessible from other similar products or related processes, and the investigations should not be constrained by likelihood, as the impact on patient health may be difficult to predict.
3) Identification of a QRM leader and any required resourcing. Any activities/outcomes need to be defined and time-bounded. For existing commercial drug products, a business case is likely to be required and identifies the reasons for change, the likely time frames, risks, and benefits [27]. In these cases, a supporting regulatory strategy would also be required, that is, a post-approval submission, identifying the knowledge space, the output of any risk assessments, and any proposed process changes (see case study 21.6.5).

21.2.2.1 QRM Process

QRM assessments start with a clear problem statement and/or risk proposal. If the risk(s) can be well defined, then a suitable risk management tool (see annex I of the guideline: "Risk Management Methods and Tools") can be identified and applied. To accurately describe the risk(s) for QRM purposes, three essential questions need to be articulated:

1) What can go wrong?
2) What is the possibility or probability that it will go wrong?
3) What are the potential consequences (i.e., what is the severity of the outcome)?

A QRM policy should be utilized throughout the life cycle of a drug product. For instance, as part of the QRM policy, "decisions on the scope and extent of validation and qualification should be based on a justified and documented risk assessment of the facilities, equipment, utilities, and processes" [28].

21.2.2.2 Risk Identification (What Can Go Wrong?)

Risk identification is a structured assessment of available data to categorize hazards relevant to the risk question or problem statement. Risks can be identified by several complimentary approaches and illustrative examples of some common approaches are provided in Table 21.2.

Table 21.2 Risk identification techniques.

Risk identification technique references	Summary
Interviews [29]	Structured interviews with experienced staff, SMEs, process operators, etc., can facilitate risk understanding. This approach is time consuming and output needs to be prioritized. This approach is best used in conjunction with other approaches
Workshops [30, 31]	Workshop discussions using cause-and-effect diagrams facilitate structured thinking and support decision making. Flowcharts break the process down into individual stages and fishbone (cause and effect) diagrams can be used to highlight the factors affecting stages and the interconnectivity between stages. Influence diagrams can be used to depict the impact of individual variables on each other and on the output
Mapping [30]	Process mapping allows the visualization of the process and assessing the risks relative to each other. The data are systematically assessed and the significance and likelihood of the risk can be evaluated
Checklists [30, 32, 33]	Checklists can be compiled based on prior experience and historical knowledge of risks obtained from comparable projects. "Prompt list" is a question-based technique for identifying risks. Checklists are a simple approach and are useful in identifying risks for new projects, but they can constrain thinking, and the potential for new risks not previously seen needs to be assessed
Delphi [29, 33]	Facilitator prepares questionnaire and SMEs and other technical experts provide feedback anonymously, which addresses human factors and any existing bias. Facilitator translates responses into risk categories, and then experts review and comment in an iterative fashion until consensus is reached
Brainstorming [32, 34, 35]	Facilitator with good leadership skills is required. Facilitator can enhance productivity by articulating objectives and scope prior to meeting. Teams need to be multifunctional with a mixture of broad-based generalists and SMEs and other process/products experts. Meetings do not pass judgment on output. Nominal group technique can remove some of bias and personal influence typically seen with standard brainstorming

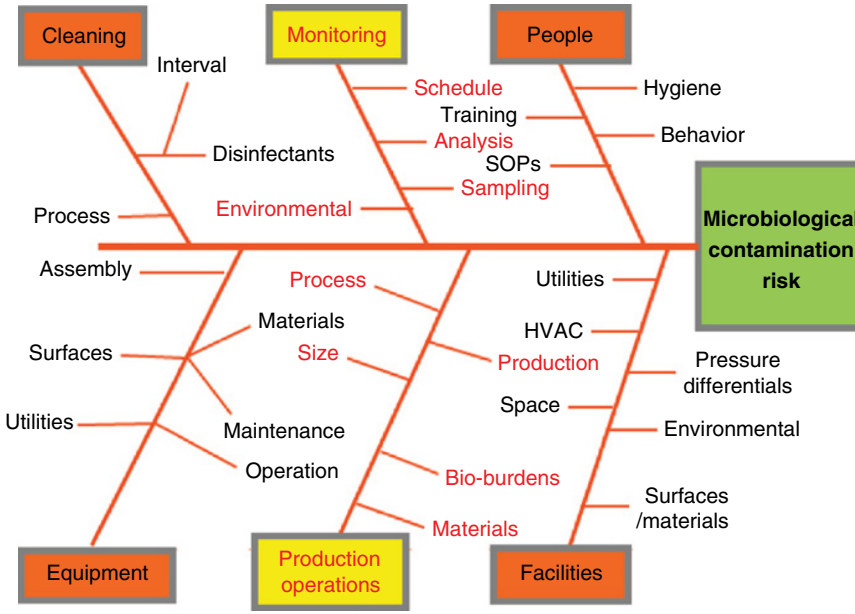


Figure 21.2 Fishbone (cause and effect) diagram highlighting potential hazards (that could lead to a microbiological contamination risks) in an aseptic process. *Source:* Adapted from Quinto and Menezes [36]. Reproduced with permission of ISPE.

An illustrative example of risk identification is provided through consideration of a manufacturing process for a sterile product. During the manufacture of sterile products, it is imperative that all processing is performed in a manner that minimizes the risk of contamination hazards [36, 37]. Consequently, this is a high risk process as the severity of harm is always going to be high and the determination of non-sterility is always of low likelihood, both from a root cause assessment and a testing perspective [37]. A typical cause-and-effect (Ishikawa fishbone) analysis of an aseptic process (see Figure 21.2) highlights the key input variables and helps to identify ways to minimize these potential hazards.

Another example relates to solid oral dosage forms. A key risk in such a dosage form is excipient incompatibility. During the development of a proposed solid oral dose formulation, an initial risk assessment should therefore facilitate identification of potential interactions between the API and formulation constituents, appropriate unit operations and any likely impact on quality attributes [38]. This would be reinforced by biopharmaceutical considerations (solubility, permeability, and dose), *in silico* predictive degradation tools such as ZENETH [39], a genotoxin (or mutagenic) risk assessment (GRA), targeted excipient compatibility studies, processing preferences, and specifications setting.

However, it needs to be clearly understood that there may be conflicting risks. For instance, selection of a solid-state form (salt, co-crystal) with higher thermodynamic activity often demonstrates higher solubility, leading to increased *in vivo* exposure; however, this also implies great chemical reactivity, leading to lower stability (both physical and chemical stability) in the solid state [40]. However, these conflicting risks can be addressed using an appropriate control strategy rather than trying to avoid (if that is indeed possible) the risk altogether.

21.2.2.3 Risk Analysis (What is the Possibility or Probability That It Will Go Wrong?)

Risk analysis is carried out to evaluate the risk(s) associated with any of the identified hazard(s), which are associated with any given process or product. The output of risk analysis can be an empirical or semiempirical process, and the intent is to compare the likelihood of occurrence of a specified hazard(s) and the severity of that hazard(s). It should be noted that in some of the QRM tools that are available, the ability to detect the harm, that is, detectability, also has an influence on the evaluation of the potential risk. For example, in a GRA, the detectability of the mutagenic (or potentially mutagenic) impurity(ies) is a significant challenge as most of these toxic impurities need to be controlled to very low ppm levels, or if the dose is very high (>1 g) to ppb levels (aligned with the TTC threshold); see ICH M7 [25]), rather than the typical ICH Q3A(R2) [23]/Q3B(R2) [24] levels, that is, 0.005% or 500 ppm [41]. Risks are then assigned a rank order using a suitable tool, for example, probability–impact matrix or similar. Perhaps understandably there are no defined standards for defining either the severity or the probability of the risk(s). This explains why there is still the perception, and sometimes the reality, that the company's perspective of the severity/probability of a given risk is not shared by the regulators [42]. However, within any given organization there should be a uniform approach to risk analysis enshrined within the company's risk master plan, itself aligned to the overall quality system. Thus, the output from any risk analysis should be retained within a risk register, which needs to be regularly reviewed and, as required, updated.

21.2.2.4 Risk Evaluation (What Are the Potential Consequences, i.e., What Is the Severity of the Outcome?)

Risk evaluation occurs after risk assessment; however, from a life cycle perspective, it is an ongoing, iterative process. Indeed, risk evaluation of a given process may occur several times throughout the lifetime of a product. During risk assessment, the known or potential risks are compared versus predetermined risk assessment criteria. Thus risk assessments can be performed using either an empirical or semiempirical approach to determine the significance of that risk. The ultimate significance of the identified risks is determined by

assessing both the impact and the likelihood of any occurrences [38]. Equally importantly, the output of the risk assessment will separate acceptable from unacceptable risks, and this in turn is linked with the tolerability of that risk in any given scenario (see Section 21.1.2). Hence, critical risks are identified, reduced to an appropriate level, and then controlled at this level, whereas other non-critical risks can be accepted with the proviso that they continue to be adequately controlled. This process should be adequately documented [43].

In performing any risk assessment, it is important to focus on the quality of the subsequent outcome, that is, the reproducibility of the available data. It is imperative to emphasize any biases or preexisting assumptions, any connection between different variables and realistic sources of uncertainty/ambiguity/error, as this will increase the reliance that can be placed on this risk evaluation and/or help to identify any underlying constraints and limitations. This ambiguity arises due to a mixture of insufficient or inadequate knowledge surrounding a key process (particularly in a multivariate system) and the likely unpredictability, for example, failure modes of a process, sources of unpredictability, and the probability of detection.

Although, the "reproducibility of the available data" is stressed within the ICH Q9 [1] guidance; there is limited information in the literature relating to this key concept. For example, if the QRM exercise was repeated with the same input variables and a second team with similar levels of expertise/experience as the original team, would they generate the same output? Or more significantly, if the exercise is repeated by regulators, will they also generate the same output? The consistency of the failure modes and effects analysis (FMEA) output from two separate QRM teams utilized during the validation of an HPLC method was assessed by Oldenhof et al. [44]. The two teams separately identified 5/6 failure modes needing urgent corrective action. However, only a third of these failure modes were commonly identified between the two teams, showing significant inconsistencies between the FMEA outputs. The authors recommended the inclusion of an experienced FMEA facilitator and the inclusion of at least two analysts having experience/competency of the method being validated (to prevent one individual's opinion from biasing the output). This issue is typically addressed in practice by having trained QRM facilitator(s) and a QRM community of practice (CoP), where best practices are shared and where ongoing assessments can be shared and collaboratively discussed, leading to a "consensus" output rather than an output that could be biased by a single SME.

Therefore, the QRM evaluation will provide either an empirical (1, 5, 10) or semiempirical (low, medium, high) risk(s) assessment. Some practitioner's use arbitrary risk scores to rank risk variables. Quantitative risk assessments are appropriate for one-factor-at-a-time evaluations, that is, univariate risk assessments. On the other hand, some QRM tools utilize a comparative risk assessment to bring together multiple levels of severity and probability into a combined estimate of relative risk, that is, multivariate risk assessments.

These semiempirical risk evaluations are quick, cheap, and reasonably easy to perform. In contrast, empirical risk evaluations take longer, are more expensive, and require trained operatives but show enhanced precision and clearer differentiation between interrelated risks and can be used to readily prioritize between those risks. The "probability of occurrence" is determined using available experience and historical precedence, both within the company and across the industry. The "probability of occurrence" is estimated semiempirically, using terms such as "high," "medium," or "low," and, empirically, with descriptive terms such as "every campaign, that is, 10-batches" or "every-batch."

21.2.2.5 Risk Control

Risk control addresses the decision-making procedures necessary to decrease risk(s) to a suitable level and/or, if appropriate, to accept risks. The total collective effort used throughout the risk control process should be in proportion to the relative importance of that risk, that is, tolerability of risk [20]. QRM teams might utilize different approaches, including risk/benefit and benefit/cost analyses, to attempt to comprehend the most favorable levels of risk control. Risk control strategy should endeavor to provide answers to the following key questions:

1) Does the risk surpass an acceptable level (either specified or defined)?
2) What strategies can be introduced to either reduce or eliminate risks?
3) What is the suitable balance between the potential benefits, apparent risks, and likely resource outlay?
4) Are there any fresh risks introduced as a consequence of the previously recognized risks being controlled or eliminated?

21.2.2.6 Risk Reduction

Risk reduction strategies focus on approaches to either mitigate or avoid quality risk(s) when they exceed a designated (specified or acceptable) limit (see Figure 21.1). The critical risks are assessed versus their recognized acceptance criteria and appropriate measures are implemented to either eliminate or mitigate these risks. A non-comprehensive list of some typical tools that have been utilized during risk reduction strategies are outlined in Table 21.3.

Risk reduction strategies could include procedures designed to alleviate the impact (or severity) and the likelihood of the specific harm. Additionally, those procedures that improve the detectability of hazard(s) or quality risk(s) may also be used as part of any risk control strategy. However, it is important to be aware that introducing risk reduction strategies can also introduce new/additional risks into this process or increase the significance of other preexisting risks. Therefore, it is relevant to revisit the risk assessment to establish and evaluate any possible changes to the risk(s) profile following any risk reduction process. For example, employing a different synthetic route could effectively

Table 21.3 Risk reduction tools.

Risk reduction techniques (references)	Summary
ALARP [45, 46]	ALARP (see Section 21.1.2) is a risk reduction/control tool based on the premise that all available efforts should be made to reduce the risk by as much as is practicably possible, but taking into account the cost/benefit ratio. Thus, risk should only be tolerated if there is a clear benefit in doing so. "Carrot" diagrams have been utilized in ALARP-driven risk assessment strategies, where unacceptable risk, tolerable risk, and acceptable risk are displayed as the top, middle, and bottom regions, respectively, of the diagram. Sometimes ALARP takes precedence over the toxicologically derived limits (i.e., ICH Q3C(R5) [47]), but in other cases, where the toxicologically derived limits constitute a virtually safe dose (VSD), that is, LTL (Less than Lifetime) (see ICH M7 [24]), the safety limits take precedence over ALARP
4Ts [48]	**Treat**, **transfer**, **terminate**, **tolerate** **Treat** the risk to avoid consequences or reduce to a "safe" level. This is typically via development of robust processes (ICH Q8(R2) [2]/Q11 [4]) and/or through the addition of additional controls. As with ALARP the efforts expended to control the risk need to be commensurate with the level of that risk. Avoidance can be practiced in vendor selection, avoiding those with poor compliance history **Transfer** the risk to a third party and it will manage the risk on your behalf; this does not eliminate the risk but can lessen its impact **Terminate** activities associated with risks that cannot be avoided or reduced **Tolerate** risk when safe levels have been achieved, for example, TTC (Threshold of Toxicological Concern) and LTL (see ICH M7 [24])
Brainstorming	See Table 21.2
CAPA [1, 43, 49]	Corrective and preventative action (CAPA) is typically applied retrospectively, but can be used proactively. CAPA approach deals with the hazards that cause the risk, implements activities to prevent recurrence and provides opportunities for continuous improvements
RCA	Root cause analysis (RCA) can be applied either reactively or proactively to the removal of risks. In addition, controls for critical steps can prevent the recurrence of risk. RCA ascribes actions, responsibilities, and time lines for completion (vs. a preassigned target date). Often used in combination with the CAPA

eliminate a specific mutagenic impurity(ies) risk, but in all likelihood would introduce some new mutagenic impurity(ies) risks that would need to be assessed and ultimately controlled [50, 51].

21.2.2.7 Risk Acceptance

Risk acceptance is a deliberate decision to accept any residual risk(s). This can either be a formalized choice to accept any residual risk(s) or may be a passive decision in which any residual risks are acknowledged, but not completely specified. For some types of hazards, even the best QRM approaches may not fully eradicate residual risks, for example, GRAs. In these scenarios, it can be argued that a control strategy has been developed and applied and that the quality risk is reduced to an acceptable, that is, predefined level, that is, the TTC or LTL, which are virtually safe doses (VSDs) (see ICH M7 [25]). This acceptance level will be dependent on many variables; it may change with time (e.g., the acceptable level for a mutagenic impurity is related to duration, less than lifetime limits (LTL) derived from the TTC decreases as the duration of the clinical study increases or as the patient dose increases) and must be assessed/reassessed when changes are made to either.

In practice, both patients and medical professionals describe the risk/benefit of a medicinal product as the "improvement that the patient can expect from taking the medicine compared with the constraints, including the undesirable effects that the patient may suffer from" [52]. Understandably, the patients' perspectives on risk/benefit are directed toward their own situation and their expectations rather than to the population as a whole. In contrast, EMA defines risk/benefit as "an evaluation of the positive therapeutic effects of a medicinal product in relation to any risk relating to the quality, safety, or efficacy of the medicinal product as regards patients' health or public health" [53]. Further, a marketing authorization shall be refused or rescinded "if the risk/benefit balance is not considered to be favorable." In practice, EMA and other competent regulatory authorities judge the risk/benefit ratio of a medicine by evaluating the outcomes of scientific assessments and studies that pharmaceutical companies perform according to internationally agreed scientific standards (ICH, WHO, EMA, FDA, etc.) to show the quality, efficacy, and safety of a medicine for obtaining a marketing authorisation [53].

The degree of risk that can be tolerated is dependent on the proximity of the risk to the patient, especially when additional "downstream" controls may be implemented after the process that is being assessed and prior to the product being delivered to the patient. Thus, an API may be extensively photolabile, for example, nifedipine [54]; however, provided that sufficient controls are in place during primary and secondary manufacturing, during the selection of the drug product container closure, etc., then the risk to the patient is minimal. For example, the primary and secondary manufacture excludes light, the product is film coated with an appropriate opacifying agent, and primary and secondary packaging can adequately protect the product throughout its designated shelf life.

21.2.2.8 Risk Communication

Communicating and appropriately documenting risk(s) to the key stakeholders within the organization (see Figure 21.1: solid arrows) and, if appropriate, to the regulatory agency (using preexisting channels) is extremely important. Typically, the information to be shared and the intended recipients are determined by the criticality of the information and any pre-agreed time lines, that is, reporting adverse incidents to regulatory agencies [55, 56]. Risk communication is the formal sharing of important information about the potential risk(s) related to the process and the ongoing QRM strategy to mitigate these risk(s). The QRM team can communicate at any stage of the process (see Figure 21.1: dashed arrows). The scope of the communication can relate to the incidence, likelihood, severity, acceptability of issue, or other aspects of QRM.

21.2.2.9 Risk Review

QRM is a fundamental part of good manufacturing practice (GMP) and the underpinning pharmaceutical quality systems (see ICH Q10 [3]). As such, a documented process to monitor/review issues must be in operation. The output of the QRM process must be reviewed periodically and must take into account any new information, change control (planned or unplanned), and other pertinent inputs.

The QRM process should continue to be used for issues and occurrences that might affect the original QRM decision. This should be irrespective of whether these processes are intentional (e.g., outcomes of drug product reviews, planned inspections, planned audits, or planned change control) or unintentional (e.g., customer complaints, recalls, CAPAs, or root cause analysis deriving from failure assessments). The timings of any reviews must be based on the likely level of risk(s). As a consequence, any risk review should include reassessment of any preceding risk acceptance decision. However, the QRM team should not be unduly biased by these previous risk assessments as knowledge gaps or a better understanding of detectability of the risk, and thereby the implementation of a control strategy can result in marked changes from preceding risk assessments (see Section 21.2.2.5).

Risk review tools, such as key performance indicators (KPIs), which monitor performance metrics or benchmarking, support and supplement the ongoing risk review process. Companies are expected to take a holistic view of QRM and to be aware that risks that impact on product quality often arise as a result of multiple failures occurring simultaneously, that is, they are additive in nature, and hence risk mitigation strategies must be sufficiently robust to minimize such eventualities [57].

Two recent product recalls both prompted by customer complaints relating to the presence of noxious odors in medicinal products, highlighting the complex multivariate nature of QRM.

In the first case study, the problem was subsequently identified as arising from accidental contamination of the product with tribromoanisole (TBA) [58].

Interestingly, TBA was not a drug product degradant, nor an extractable from the container closure system, but was identified as a volatile contaminant arising from the wooden pallets that were used to ship the product to the wholesaler. The wooden pallets had been sprayed with a fungicide to promote longer life, but the fungicidal agent had subsequently degraded to form TBA, which due to its high volatility could easily migrate across the high density polyethylene (HDPE) containers into the product in sufficient quantities to be discernable by the patient (often occurring at ppt levels). This issue was easily addressable by substituting stainless steel pallets for the wooden ones.

In the second case study, the problem was subsequently identified as arising from accidental contamination of a holding tank containing methane sulfonic acid (the counterion used during salt formation of the API) with ethanol [59]. The ethanol had been introduced as part of the routine cleaning procedures for the holding tank. Unfortunately, significant residual levels of ethanol remained within the tank after cleaning, and they reacted with the methane sulfonic acid to form the corresponding mutagenic ester, ethyl methane sulfonic acid (EMS). Residual ppm levels of EMS were carried through to the final drug product. The conditions necessary for formation of sulfonate ester require strongly acidic conditions [60] that are absent in normal manufacturing procedures used to produce sulfonic acid salts of the active; however under the specific/unique conditions experienced in the head tank, levels of EMS were formed. This issue was easily addressable by removing the holding tank from the primary process and introducing controls into the specification for the raw materials and into the API process and specification. These somewhat extreme examples highlight the need to assess all parts of the supply chain as part of QRM initiatives.

21.2.2.10 Risk Management Methodology

QRM is at the center of any science-based or practical approach to risk-based decision making. QRM ensures that there is a transparent, consistent, and documented approach for the individual stages of the QRM process. Historically, quality risk(s) have been assessed and controlled using a selection of informal approaches (quantitative, semiquantitative, qualitative, and/ or company risk-based processes), typically involving review of existing data/ knowledge, batch data trending, etc. Obviously, such strategies will continue to provide relevant and useful information that will support ongoing GMP initiatives. However, going forward, it benefits both industry and regulatory agencies to have a QRM process, whereby quality risks can be managed in a transparent manner using widely accepted risk management tools, as well as established internal GMP processes. There are a wide range of different approaches available for risk analysis. A summary of these different approaches and their potential advantages and disadvantages is provided in Table 21.4.

It may be appropriate to modify these approaches or use different tools, which are more appropriate to API, drug product, or device issues. QRM tools

Table 21.4 Pros and cons of principal risk-based tools.

Risk analysis tool	Pros	Cons	Comments
Control chart, Pareto chart, Shewart chart, etc.	Simple approaches. Limited resource and training required. Easy to generate, using widely available tools, for example, Microsoft Excel. Trends, patterns, and process control easily visualized. Can be used with ALARP for assessing risk tolerance levels	Cannot be used "as is" (requires additional risk analysis tools) Measurement method can bias results. Identifies process variability or process changes, but does not identify cause. Needs a statistically significant number of data points	Charts are statistically based and routinely used in production/QC to demonstrate "process control." Useful ancillary tool to assess risks associated with process(es) and suppliers
PHA [1] (preliminary hazard analysis)	Requires limited data (can be used early in development process). Limited resource and training required. Systematically identifies and ranks risk. Visual overview of failure modes	Typically, only identifies major hazards (minor hazards may be missed). Hazard interaction is difficult to determine. Bias can be introduced during selection of filter levels. Typically a preliminary assessment	Used with other risk tools. Severity and probability of risks are assessed. Applicable to product, process and facility design
FTA [1] (fault tree analysis)	Visual overview of failure modes. Causes can be logically assessed. Wide range of factors causing failure can be identified. Generates useful process data (relevant to future state)	Requires extensive data, and is expensive and time consuming. Requires significant training and expertise to generate useful outputs. Relies on deep process understanding. Assess failures "one factor at a time;" it has some ability to look at multivariate issues	Uses logic gates to identify hazards. Graphically presents the hazards. Can be used to assess complex processes. Top-down process. Applicable to change control, deviations, process and process design, customer complaints, and commissioning

(*Continued*)

Table 21.4 (Continued)

Risk analysis tool	Pros	Cons	Comments
FMEA [1] (failure mode effect analysis)	Can be used "as is" or with other risk tools. Risk priority number (RPN) is useful in ranking risks. Minimizes unforeseen failures. Continuous process (follow-up system available to reevaluate hazards). Can introduce immediate remediation for actual risks or a remediation strategy for potential risks	Requires extensive data and is expensive and time consuming. Requires significant training and expertise to generate useful outputs. Relies on deep process understanding. Errors can occur in interpreting output. The three input variables for RPN are not equally weighted (likelihood and detectability are inversely correlated)	Identifies possible hazards and impact on performance. Bottom-up process. RPN is derived by multiplying severity, probability, and detectability. Risk reduction strategies utilized to reduce RPN to as low as possible. Applicable to change control, process and process design, and commissioning
HACCPs [1] (hazard analysis and critical control points)	Can assess complex scenarios, where hazards are diverse and which involve large data sets. Rapid detection of issues possible. The accrued data can be useful for future applications (both in risk assessment and control). Rapidly identifies critical control points	Expensive and time consuming. Requires significant training and expertise to generate useful outputs. It has to be utilized with other tools to assess risks fully. Typically used for evaluation of manufacturing processes and contamination	Based on seven unique principles: (i) hazard analysis, (ii) determine critical control points (CCPs), (iii) establish target and critical limits, (iv) monitor CCPs, (v) establish corrective action to regulate CCPs (if required), (vi) establish verification process to prove that HACCP is working, and (vii) document. Applicable to process and process design, suppliers, and commissioning

Source: Adapted from Charoo and Ali [38]. Reproduced with permission of Taylor & Francis.

and the supporting statistical assessments can be utilized in combination, for example, probabilistic risk assessment (PRA). PRA models can be used to assess the frequency and consequences of not achieving a safe, reproducible end state. PRA provides intrinsic flexibility that can facilitate the role of QRM in a quality system. As previously indicated, the degree of rigor of the process and the level of documentation of the QRM should mirror existing information and knowledge and be aligned with either the complexity and/ or criticality of the risk issue identified.

21.3 QRM and GMP

QRM is also covered by GMP inspection processes, and it is considered an integral part of chapter 1 of the EU GMP guidelines [57]. As such, all marketing authorization holders (MAHs), third-party manufacturing sites, API manufacturers, etc., must have a QRM system in place. Thus regulatory inspectors will review QRM as part of the quality systems section [3] (of any inspections, which also includes product quality reviews, complaints, recalls, and deviations). In addition, inspection teams may review those risk assessments that are germane to the inspection. Finally, based on an inspectors' interpretation of the significance of the risk to any ongoing manufacturing operations, they may request that the company produces a formal summary of the risk assessment, any key decisions arising from that assessment, and the supporting conclusions [42, 57].

21.4 Integration of QRM into Industry and Regulatory Operations

It is expected that introduction of QRM within an overall quality system (see ICH Q10 [3]) will support better science-based risk decision-making, As previously articulated, the appropriate use of QRM does not negate or circumvent industry's obligation to comply with regulatory requirements, including GMP. But equally, importantly, ICH Q9 [1] indicates that "these examples are not intended to create any new expectations beyond the requirements laid out in the current regulations." It is anticipated that QRM will reduce subjectivity by using data-driven approaches that focus on the highest identified risks, support prioritization of resources, and ultimately provide regulators with greater assurance of a company's ability to manage potential risks. In principle, this directly affects the level and overall extent of direct regulatory oversight, that is, a reduction in the "regulatory burden." Thus, a company can significantly reduce the number and related costs of post-approval submissions and manage the timings of any subsequent filings. This is mirrored by a desire from the regulatory agencies to revise existing guidance; in the EU the acknowledgment of the

need to change their guidance on variations and change controls [61] and in the United States the desire to modify post-approval regulations [62]. Although there are compelling drivers supporting moves to adopt QRM-based initiatives, the very real barriers to change for established products need to be understood. Under the banner of "if it isn't broke, why fix it?," these obstacles include (i) a very real reluctance to make changes to pharmaceutical processes that are operating in a satisfactory manner, (ii) a reluctance to initiate changes requiring regulatory oversight, and (iii) perceptions that risk/benefit for change is not well understood, that is, the efforts required may outweigh the benefits [27, 63].

Appropriate and adequate training of key personnel (from both industry and regulators) in a QRM process will ensure greater understanding of risk-based decision-making processes and will build mutual confidence in QRM outcomes. Indeed this is an absolutely vital undertaking and without that shared understanding of decision making, risks can often reside in the "eye of the beholder" [42].

21.5 ICH Q9 and Other QRM Initiatives

ICH Q9 [1], ICH Q8(R2) [2], ASTM (American Society for Testing and Materials) standard E 2500-0762, International Society of Pharmaceutical Engineering (IPSE) new risk-based approach (Risk-MaPP) to validation and verification [64], and PDA (Parenteral Drug Association) Quality Risk Management for Asceptic Products [37] all share many common features:

1) QRM is the key underpinning strategy supporting the QC strategy for any product in development or manufacturing.
2) Risk analysis, risk assessment, and risk management are essential requirements.
3) Utilization of prior knowledge is important in defining the product, process, and facility.
4) There needs to be deep understanding of both the product and supporting process with regard to fundamental mechanistic properties rather than empirical understanding, that is, supported by experience and observation rather than logic or theory.

21.6 Case Studies

21.6.1 Introduction

The PhRMA Drug Product Technology Group (DPTG) published an overview of the role of risk management in the drug development process [65]. They repeatedly stressed the importance of ensuring "acceptable risk versus no risk," and highlighted that "overly conservative control of quality may result in

wasteful applications of time and resources in the development and manufacture of drug products, directly contradicting the mission enunciated above in the FDA's strategic action plan" [66]. They outlined four different approaches that can be anticipated from any risk assessment evaluation: (i) acceptable risk, with no additional assessment required; (ii) potential risk, with additional assessment required; (iii) significant risk, with a well-defined, risk management strategy (RMS) being typically required; and (iv) unacceptable risk, which will typically require significant investigation, reengineering, or even in extremis, abandonment of the proposed process or product.

21.6.2 Risk Assessment of an API Design Space: Impact of Scale

It has been proposed that

> it might be possible to conduct verification studies of the performance of the design space scale-dependent parameters as part of process validation. A risk-based approach can be applied to determine the design of any appropriate studies for assessment of a design space across different scales. [4]

There has prompted some significant concern within the pharmaceutical industry as to whether design spaces, which for the most part have been developed and verified within an R&D setting, at pilot scale, can be or indeed should be reestablished at commercial scale, where the cost would be prohibitive and the benefits nebulous [67]. A typical example of the type of regulatory question, reflecting differing interpretations of the underpinning ICH guidance (ICH Q8 [2], Q9 [1], and Q10 [3]), was reported by Watson et al. [67]:

> The established design space should still be verified on production scale. It is not expected that the design space is challenged near the edge of failure, but a suitable set of experiments should be performed on full production scale, and the results should be compared with the outcome that is predicted by the design space.

The authors proposed an innovative approach to address this question that was subsequently endorsed by the regulators. The authors indicated that design space boundaries (or edge of failure) are characterized by proven acceptable ranges (PARs), but that the process is actually operated within a subset of the PAR, the so-called normal operating range (NOR). They indicated that any proposed change to the registered manufacturing process within the design space needs to be assessed as part of change control, control strategy, and the quality attributes of the API or drug product. This assessment needs to include structured risk as assessment, as per ICH Q9 [1].

Clearly, risk assessments and process modeling should assess the potential impact of both scale and equipment type. Prior process experience and literature precedent can be used to initially identify and assess scale-dependent risk. For API processes, kinetic, thermodynamic, process modeling allied with knowledge of scale-dependent equipment are key to identifying and understanding scale dependency. For instance, crystallization and isolation show high scale and equipment dependencies [68]. Equally important is to identify those factors that are independent of scale so that clear conclusions can be made as to whether or not any further investigations are needed at scale.

21.6.3 Risk Assessment of a Drug Product (a): Wet Granulation Process

The factors affecting the wet granulation manufacture of Ramipril tablets were evaluated by Aksu et al. [69]. A five-level hierarchical DoE (Design of Experiments) consisting of 128 experiments were interrogated using FMEA to understand the effects of two proposed formulation changes and three process variables. Both lubricant type (sodium stearyl fumarate or magnesium fumarate) and lubricant level (0.6–1.2% and 0.75–1.0%, respectively) were assessed. The process variables studied were sieve size (0.8 and 1.25 mm), residual moisture levels (0.5 and 1.0%), and drying temperature (50 and 60°C). Then risk quantitation was determined by ranking severity (S), probability (P), and detectability (D) in the range 1–5 for process variables and formulation changes. Risk priority number (RPN) was used to assess relative risk, which is determined by multiplying S, P, and D. RPN was categorized as low (1–45), medium (46–90), or high (91–125) values, and those variables with high RPN values were deemed critical. Based on these values, lubricant type and binder concentration were deemed to be critical formulation components, and tablet hardness, disintegration, and dissolution times were determined to be critical quality attributes (CQAs). Although the authors claimed that impurities A and B were also CQAs, they indicated that "they were not monitored as CQAs" and that "because impurity A and impurity B had a value of zero in all analyses, they have not been regarded as a source of risk." It is difficult to understand how an attribute that is not formed can be a CQA unless it poses a risk to patients, that is, a mutagenic impurity (see ICH M7 [25]); but in this case this is clearly not the case as it is controlled in accordance with ICH Q3B(R2) [24], that is, 0.5%. The critical process parameters (CPPs) were determined to be percent residual moisture, drying temperature, and sieve size.

21.6.4 Risk Assessment of a Drug Product (b): Blending Process

Kapsi et al. [70] developed an orthogonal design space for the blending process of a film-coated tablet. They conducted a detailed risk assessment as per ICH Q9 [1] of all possible failure modes for this process using a combination of input process output (IPO), critical relationships matrices (CRM), and FMEA.

Each unit operation was assessed using IPO to understand and identify attributes for all input materials, assess material movement across this process, and finally identify all key attributes of output material. The IPO assessment was used to generate CRMs for each part of the process. In particular the effect of variability in the process and in key input components on the output material attributes was assessed. FMEA was then carried out, assessing all of the potential failure modes for all unit operations. Each was ranked according to the severity of that failure, and its impact on the quality of the resultant drug product (and particularly the CQAs), the likelihood of that occurrence happening and the ability to detect that failure. This was done using a scoring system for all of these risk assessment variables, that is, severity (S), likelihood (L), and detectability (D), of between 1 and 10. Then the combined effect of these individual risk assessment variables was generated, that is, a RPN and categorized by rank order. Finally, those critical parameters that required assessment and optimization were picked according to the Pareto 80:20 rule, that is, approximately 80% of the effects come from 20% of the causes. Based on the output from the FMEA assessment, they utilized DoE approaches to investigate the four key manufacturing variables that were found to significantly impact on drug product CQAs: blend volume, mixing speed, blend mixing time, and lubricated blend mixing time. The effect on each of these four attributes measured using response surfaces was assessed statistically using analysis of variance (ANOVO), and a design space for the appropriate drug product CQAs was produced using 95% predicted interval estimates. Finally, an orthogonal design space for this blending process was developed by superimposing the individual design spaces generated for each individual drug product CQA. Thus by utilizing QRM principles, significant manufacturing flexibility was introduced into the blending process.

21.6.5 Risk Assessment of a Drug Product (c): Removal of a Terminal Sterilization Process from a Non-Sterile Intranasal Product

Potter [27] reported on the redesign of an approved single-use intranasal drug product. The authors looked to assess the opportunities for replacing the existing terminal sterilization unit operation with an asceptic filtration step and the impact on the registered acceptance criteria for the supporting CQAs, in particular, the microbial limit test. An overview of the proposed change indicated that in addition to the microbial limit test, other CQAs that could be affected were appearance, pH, assay, impurities, color, and delivered volume. Whereas the proposed change may impact adversely on the microbial limit test (CQA), results from 14 commercial batches manufactured over a 6-month period indicated a positive impact on other CQAs, particularly impurities. Autoclaved product had total impurities in the range 1.1–1.2%; however, in contrast aseptically filtered batches had total impurities in the range 0.8–0.9%.

The authors assessed the microbiological controls of the existing process and showed that aseptic filling, vial filling under laminar airflow, environmental monitoring, and bio-burden of bulk solution provided a good level of control. They could link this with the microbial purity of the product pre-autoclaving and demonstrated that the bio-burden of 50 commercial batches manufactured over a 5-year period showed results of <1 cfu/ml (vs. a specification limit of ≤100 cfu/ml). In addition the product was shown to be inherently bactericidal, as well as being fungistatic, and could readily comply with the existing USP antimicrobial effectiveness testing (AET) procedure and demonstrated a 3-log reduction of the challenge organisms. Therefore, the risk to patient safety of removing the autoclaving step was deemed to be minimal. The company assessed the risk using failure mode, effects, and criticality analysis (FMECA). The severity, occurrence, and detectability of the proposed change were then assessed, and this gave an RPN value 105 (only severity was different, having a score of 5× greater for the proposed change) versus 21 for the existing process. Based on the predefined risk strategy, risks that are ≥105 pose a risk to patient's health; in this case a risk that potentially contaminated vials or stoppers could affect product quality. A corrective action program was identified (amend process equipment design, assess/increase the supporting environmental monitoring program, tighten microbiological specifications for critical components, enhance cleaning and sanitation systems, and modify supporting standard operating procedures (SOPs)) and implemented, which lowers the rank order of the risk to 25. This correlates with very low risk to patient.

The biggest challenge from the company's perspective was translating the output of the risk assessment into a regulatory submission. Post-approval submissions, including the underpinning knowledge based on product and process, together with FMECA output were subsequently submitted and approved by European and US regulators with minimal delay and limited questioning [27].

21.6.6 Stability Risk Assessment

Stability risk assessment (SRA) has been pioneered principally by Pfizer [71]. SRA utilizes existing knowledge to identify what aspects of stability need further study or monitoring. SRAs help to identify the stability-related quality attributes (SRQAs) and the shelf-life limiting attributes (SLLAs), which are based on the quality target product profile (QTPP) of a product with a shelf life of 24 months when stored at 15–25°C. The SLLAs are assessed using a QRM risk assessment approach outlined in Table 21.5.

A typical example is provided in Table 21.6, for a product early in clinical development. When a combination of input variables and/or attributes resulted in a high SLLA, then these are designated with dark shading. Moderate/unknown risks are designated as having medium SLLAs (medium shading),

Table 21.5 Rank ordering of shelf-life limiting attributes (SLLA).

SLLA definition	Risk ranking
Existing data/literature suggests that this attribute will not limit product shelf life	3
Existing data/literature suggests that this attribute may limit product shelf life, where (i) existing data "predict" some sensitivity, and/or (ii) literature "suggests" potential impact. More data are required to provide an unambiguous prediction	5
Existing data/literature suggests that this attribute will limit product shelf life; based on (i) actual stability data, (ii) *in silico* predictions based on actual data, or (iii) literature is strongly suggestive that there is a known impact, using similar structural motifs	10

Source: Adapted from Colgan et al. [71]. Reproduced with permission of Springer.

Table 21.6 SRA output.

	Physical stability	Chemical stability			
SLLAs	Appearance	Chiral purity	API potency	Degradation product 1	Total degradation
Specification acceptance criteria	Clear, colorless to yellow-colored solution	NMT 0.2%	90.0–110% Label claim	NMT 0.5%	NMT 2.0%
Ability to impact SLLA	10	1	7	10	10
Stability parameter					
Polymorph	5	1	1	1	1
Solvent levels	1	1	1	1	1
Metal content	5	5	5	5	5
In-use operations	5	1	1	1	1
Light	10	10	10	10	10
Oxygen	5	5	5	5	5
Accelerated temperature	10	1	1	10	10
Long-term temperature	5	1	1	5	5

Source: Adapted from Colgan et al. [71]. Reproduced with permission of Springer.

whereas low risks are designated as having low SLLAs (no shading). The SRA table clearly identifies those attributes likely to become high SLLAs (appearance, degradation product 1, and total degradation products). For example, one mitigation strategy would be to minimize light exposure using appropriate modifications to the secondary process and using appropriately protective primary (amber glass vials) or secondary (light protective cardboard inner and outer) packaging.

21.7 Conclusion

Although QRM "appears" to be a relatively new concept, it has been used in many other industries for many decades [72]. Historically, the pharmaceutical industry has been slow to adopt these "risk-based" procedures because regulatory and GMP compliance (as defined by international regulatory agencies, that is, FDA, EMA, PMDA, etc.) were seen as the principal arbiters of pharmaceutical quality. But it has become clear to all parties that a "one-size-fits-all," compliance-based approach will not be appropriate for the 21st century. The FDA initially initiated a quality systems approach [73], and this has been subsequently embedded into the ICH process with the implementation of ICH Q8(R2) [2], Q9 [1], and Q10 [3]. QRM has been applied to many different areas of the pharmaceutical industry. A non-exhaustive list would include assisting facility design; qualification of facilities, processes, manufacturing equipment, and laboratory methods; qualification of product development activities (including identification of CQAs and CPPs/appropriate specifications); determination of acceptable cleaning limits; impact of change control; computer validation; etc. [72].

ICH Q9 [1] indicates that the evaluation on quality risks should be based on scientific knowledge and that the level of effort and subsequent documentation of the resultant process is commensurate with the level of risk. The process is subdivided into the following areas:

- Risk assessment (including identification and evaluation)
- Risk control
- Risk reduction
- Risk acceptance
- Risk communication
- Risk review

ICH Q9 [1, appendix 1] provides a nonproscriptive list of appropriate QRM tools that can be used as part of the risk-based process. These tools aim to provide the user with a series of qualitative, semiquantitative, or quantitative outputs that can be useful in identifying and controlling development risks. Although the outputs from these QRM tools are fully documented and

transparent in nature, they are not always fully accepted by the regulatory community, and the old adage that "risk is in the eyes of the beholder" [42] does certainly appear to be true. In addition, these tools do not appear to be reproducible in nature and different outcomes are possible from the same input parameters [44]. The PhRMA DPTG highlighted the role of risk management in the drug development process [65]. They stressed the importance of ensuring that there was a tolerability of risk, that is, "acceptable risk versus no risk."

Several case studies were included to provide the reader with relevant and recent examples of how QRM has been used in the following areas:

- Risk Assessment of an API Design Space including the impact of scale-up [67]
- Risk Assessment of a Drug Product (a) Wet Granulation Process [69]
- Risk Assessment of a Drug Product (b) Blending Process [70]
- Risk Assessment of a Drug Product (c) Removal of a terminal sterilization process from a non-sterile intranasal product [27]
- Stability Risk Assessment (SRA) [71]

These serve to illustrate the utility and value of the use of QRM tools in the successful development of robust products that assure the quality and integrity of the final product and why such tools form an integral part of an overall quality and design-driven development process.

References

1 ICH Q9, 2005. Quality risk management. Current step 4 version, November 9, 2005.
2 ICH Q8(R2), 2009. Pharmaceutical development. Current step 4 version, August 2009.
3 ICH Q10, 2008. Pharmaceutical quality system. Current step 4 version, June 4, 2008.
4 ICH Q11, 2012. Development and manufacture of drug substances (Chemical Entities and Biotechnological/Biological Entities). Current step 4 version, May 1, 2012.
5 Pirsig RM, 1974. *Zen and the Art of Motorcycle Maintenance*. William Morrow, New York.
6 Korakianti E, Rekkas D, 2011. Statistical thinking and knowledge management for quality-driven design and manufacturing in pharmaceuticals. *Pharmaceutical Research* 28; 1465–1479.
7 Montgomery DC, 2001. *Introduction to Statistical Quality Control*. John Wiley & Sons, Inc., New York.
8 Deming WE, 1988. *Out of Crisis*. Center for Advanced Engineering Study, MIT Press, Cambridge, MA.
9 ASQ. Glossary. http://asq.org/glossary/q.html. Accessed on January 20, 2014.

10 Hoyer RW, Hoyer BBY, 2001. What is quality? *Quality Progress*; 53–62. http://asq.org/search?proxystylesheet=sitewide&client=sitewide&getfields=Price.xStandardStatus.xCopyrightHolder.CoverURL.xShortDescription.xJournalName.dDocTitle.xFormat.xItemNumber.dDocType.xCreator.xAbstract.xPublishDate.xCopyrightYear.xStandardDesignation&sort=date:D:L:d&filter=0&site=public&q=Hoyer. Accessed January 20, 2014.

11 ICH Q6A, 1999. Specifications: Test procedures and acceptance criteria for new drug substances and new drug products: Chemical substances. Current step 4 version, October 6, 1999.

12 Elder DP. 2015. The cost of drug counterfeiting. *European Pharmaceutical Review* 20(1); 5.

13 Elder DP, Kuentz M, Holm R, 2016. Pharmaceutical excipients—Quality, regulatory and biopharmaceutical considerations. *European Journal of Pharmaceutical Sciences* 87; 88–99.

14 Rentz ED, Lewis L, Mujica OJ, Barr DB, Schier JG, Weerasekera G, et al. 2008. Outbreak of acute renal failure in Panama in 2006: A case-control study. *Bulletin World Health Organization* 86(10); 749–756.

15 WHO, 2006. Annex 4. Supplementary guidelines on good manufacturing practices: Validation. WHO Technical Report Series, No. 937. http://www.who.int/medicines/areas/quality_safety/quality_assurance/SupplementaryGMPValidationTRS937Annex4.pdf. Accessed on January 25, 2014.

16 Woodcock J, 2004. The concepts of pharmaceutical quality. *American Pharmaceutical Review* 7; 10–15.

17 FDA, 2007. FDA says consumers continue to buy risk drugs online. http://www.health.am/ab/more/fda-says-consumers-continue-to-buy-risky-drugs-online/. Accessed January 25, 2014.

18 Cogdill RF, Drennan JK, 2008. Risk-based quality by design (QbD): A Taguchi perspective on the assessment of product quality, and the quantitative linkage of drug product parameters and clinical performance. *Journal of Pharmaceutical Innovation* 3; 23–29.

19 Food Additive Amendment of 1958, Section 409(c)(3)(A).

20 Bouder F, 2008. Regulating impurities in pharmaceutical products: A tolerability of risk approach? *Expert Reviews in Clinical Pharmacology* 1; 241–250.

21 McQuaid D, 2007. An historical perspective of tolerability of risk. *In*: *The Tolerability of Risk: A New Framework for Risk Management*. Bouder F, Slavin D, Löfstedt D (Eds), Earthscan, London.

22 ICH S9, 2009. Nonclinical evaluation for anticancer. Current step 4 version, October 29, 2009.

23 ICH Q3A(R2), 2006. Impurities in new drug substances. Current step 4 version, October 25, 2006.

24 ICH Q3B(R2), 2006. Impurities in new drug products. Current step 4 version, June 2, 2006.

25 ICH M7, 2014. Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. Current step 4 version, June 23, 2014.
26 Delaney EJ, 2007. An impact analysis of the application of the threshold of toxicological concern concept to pharmaceuticals. *Regulatory Toxicology and Pharmacology* 49; 107–124.
27 Potter C, 2009. PQLI Application of science- and risk-based approaches (ICH Q8, Q9, and Q10) to existing products. *Journal of Pharmaceutical Innovation* 4; 4–23.
28 Eudralex Volume 4, EU Guideline for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use. Annex 15: Qualification and Validation, Brussels, March 30 2015. http://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2015-10_annex15.pdf. Accessed on May 5, 2017.
29 Elkington P, Smallman C. 2002. Managing Project risks: A case study from the utilities sector. *International Journal of Project Management* 16; 333–343.
30 Planning a Workshop: Organizing and Running a Successful Event. https://www.mindtools.com/pages/article/PlanningAWorkshop.htm. Accessed on May 5, 2017
31 Ishikawa K, 1991. Introduction to quality control. 3A Corporation, Japan.
32 Chapman C, Ward S, 1997. *Project Risk Management. Processes, Techniques and Insights*. John Wiley & Sons, Ltd., Chichester, p. 322.
33 Chapman RJ, 1998. The effectiveness of working group risk identification and assessment techniques. *International Journal of Project Management* 16; 333–343.
34 PMI (Project Management Institute), 2000. *A Guide to a Project Management Body of Knowledge*. PMI, Upper Darby, PA, p. 322.
35 Githens GD, Peterson RJ, 2001. Using risk management in the front end projects. *In*: Proceedings of the Project Management Institute, 32nd Annual Seminars and Symposium, Nashville, Tennessee, November 1–10, 2001.
36 Quinto A, Menezes JC, 2010. Design, validation and control of sterile manufacturing facilities: A brief overview from the perspective of risk management and existing regulations. *Pharmaceutical Engineering* 30; 1–9.
37 PDA, 2008. Quality risk assessment for aseptic processes, PDA Technical Report No. 44. www.pda.org. Accessed on April 9, 2017.
38 Charoo NA, Ali AA, 2013. Quality risk management in pharmaceutical development. *Drug Development and Industrial Pharmacy* 39; 947–960.
39 Zeneth, 2016. http://www.lhasalimited.org/products/zeneth.htm. Accessed on May 10, 2016.
40 Elder DP, Patterson, JE, Holm, R, 2013. The solid-state continuum: A perspective on the interrelationships between different solid-state forms in drug substance and drug product. *Journal of Pharmacy and Pharmacology* 67; 757–772.

41 Elder DP. 2010. Genotoxic impurities, analysis of genotoxic impurities, review of approaches. *In*: *Genotoxic Impurities: Strategies for Identification and Control*, Teasdale A, Ed., John Wiley & Sons, Ltd, London, pp. 249–280.

42 Elder DP. 2014. Foreword: Is risk in the eye of the beholder? *European Pharmaceutical Review* 19(2); 7.

43 Jenkins J, Ahern J, Cock D, Shutler S, Smalley R, Hooper S. 2010. A guide to supply chain risk management for the pharmaceutical and medical device industries and their suppliers. The Chartered Quality Institute. http://www.thecqi.org/Community/Special-Interest-Groups-SIGs/Pharmaceutical-Quality-Group/PQG-guide/. Accessed on February 10, 2014.

44 Oldenhof MT, van Leeuwen JF, Nauta MJ, de Kaste D, Odekerken-Rombouts YMCF, Vredenbregt MJ, et al. 2011. Consistency of FMEA used in the validation of analytical procedures. *Journal of Pharmaceutical and Biomedical Analysis* 54; 592–595.

45 Martin CK, Whitby M. 2003. Application of ALARP to extremity doses for hospital workers. *Journal of Radiological Protection* 23; 405–421

46 Smith K. 2009. *Environmental Hazards: Assessing Risk and Reducing Disaster*. Routledge, London, p. 67.

47 ICH Q3C(5). 2011. Impurities: Guidelines for residual solvents. Current step 4 version, February 4, 2011.

48 Hopkin P. 2012. *Fundamental of Risk Management*, 2nd Edition. Kogan Page, p. 225. http://my.safaribooksonline.com/book/-/9780749465391/21-tolerate-treat-transfer-and-terminate/225#X2ludGVybmFsX0J2ZGVwRmxhc2hSZWFkZXI/eG1saWQ9OTc4MDc0OTQ2NTM5MS8yMjY=. Accessed on February 11, 2014.

49 WHO, 2006. Annex 4. Supplementary guidelines on good manufacturing practices: Validation. WHO Technical Report Series, No. 937. http://www.who.int/medicines/areas/quality_safety/quality_assurance/SupplementaryGMPValidationTRS937Annex4.pdf. Accessed on January 25, 2014.

50 Teasdale A, Elder DP, Chang S-J, Wang S, Thompson R, Benz N, et al. 2013. Risk assessment of genotoxic impurities in new chemical entities: Strategies to demonstrate control. *Organic Process Research and Development* 17; 221–230.

51 Elder DP, Teasdale A. 2015. Is avoidance of genotoxic intermediates/impurities tenable for complex, multi-step syntheses? *Organic Process Research and Development* 19(11); 1437–1446.

52 EMA, 2009. Information on benefit-risk of medicines: Patients', consumers' and healthcare professionals' expectations. EMEA/40926/2009. London, June 23, 2009. http://www.ema.europa.eu/docs/en_GB/document_library/Other/2009/12/WC500018433.pdf. Accessed on January 25, 2009.

53 EMA, 2001. Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the community code relating to medicinal

products for human use Article 1 point 28 of Directive 2001/83/EC as amended. http://www.coe.int/t/dghl/standardsetting/medicrime/dir2001_83_cons_20081230_en.pdf. Accessed on January 25, 2014.

54 Grundy JS, Kherani R, Foster RT. 1994. Photostability determination of commercially available Nifedipine oral dosage formulations. *Journal of Pharmaceutical and Biomedical Analysis* 12; 1529–1535.

55 FDA, 2009. Guidance for clinical investigators, sponsors, and IRBs adverse event reporting to IRBs — Improving human subject protection. U.S. Department of Health and Human Services, Food and Drug Administration Office of the Commissioner (OC), Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), Center for Devices and Radiological Health (CDRH) Office of Good Clinical Practice (OGCP), January 2009, Procedural

56 EMA, 2015. Reporting requirements of Individual Case Safety Reports (ICSRs) applicable to marketing authorization holders during the interim period. June 29, 2015 EMA/411742/2015 Rev. 9 Inspections and Human Medicines Pharmacovigilance Division.

57 MHRA. 2013. Annex 8: Quality Risk Management (QRM). Frequently asked questions.

58 Huang J-T, Alquier L, Kaisa JP, Reed G, Gilmor T, Vas G. 2012. Method development and validation for the determination of 2,4,6-tribromoanisole, 2,4,6-tribromophenol, 2,4,6-trichloroanisole, and 2,4,6-trichlorophenol in various drug products using stir bar sorptive extraction and gas chromatography–tandem mass spectrometry detection. *Journal of Chromatography A* 1262; 196–204.

59 Gerber C, Toelle H-G. 2009. What happened: The chemistry side of the incident with EMS contamination in Viracept tablets. *Toxicology Letters* 190; 248–253.

60 Teasdale A, Eyley SC, Delaney E, Jacq K, Taylor-Worth K, Lipczynski A, et al. 2009. Mechanism and processing parameters affecting the formation of methyl methane sulfonate from methanol and methane sulfonic acid: An illustrative example for sulfonate ester impurity formation. *Organic Process Research and Development* 13(3); 429–433.

61 Annex 15: Qualification and Validation, Section 11 Change Control, 2015. EudraLex Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use, March 30, 2015.

62 FDA. 2010. Guidance for industry CMC post-approval manufacturing changes reportable in annual reports, March 2014. https://www.fda.gov/downloads/Drugs/…/Guidances/UCM217043.pdf. Accessed on May 5, 2017.

63 ASTM E2500-13. 2013. Standard guide for specification, design, and verification of pharmaceutical and biopharmaceutical manufacturing systems and equipment, ASTM International, West Conshohocken, PA, 2013, www.astm.org. Accessed on May 5, 2017.

64 IPSE. 2011. ISPE good practice guide: Applied risk management for commissioning and qualification. http://www.ispe.org/ispe-good-practice-guides/applied-risk-management-cq. Accessed on January 12, 2014.
65 Hulbert MH, Feely LC, Inman EL, Johnson AD, Kearney AS, Michaels J, et al. 2008. Risk management in the pharmaceutical product development process. *Journal of Pharmaceutical Innovation* 3; 227–248.
66 FDA, 2003. FDA's strategic action plan protecting and advancing America's health: Responding to new challenges and opportunities. http://www.nti.org/media/pdfs/13_1.pdf?_=1316466791. Accessed on January 20, 2014.
67 Watson TJN, Bonsignore H, Callaghan-Manning EA, Colgan ST, Fitzsimons P, Garcia TP, et al. 2013. A design space verification for a small molecule drug substance. *Journal of Pharmaceutical Innovation* 8; 67–71.
68 Burcham CL, Jarmer DJ. 2013. Industrial crystallization of pharmaceuticals: Capability requirements to support an outsourcing paradigm. *Pharmaceutical Outsourcing*, September 25, 2013.
69 Aksu B, Paradkar A, de Matas M, Özer Ö, Güneri T, York P. 2013. A quality by design approach using artificial intelligence techniques to control the critical quality attributes of Ramipril tablets manufactured by wet granulation. *Pharmaceutical Development and Technology* 18; 236–245.
70 Kapsi SG, Castro LD, Muller FX, Wrzosek TJ. 2012. Development of a design space for a unit operation: Illustration using compression-mix blending process for the manufacture of a tablet dosage form. *Journal of Pharmaceutical Innovation* 7; 19–29.
71 Colgan ST, Timpano RJ, Roberts M, Weaver R, Ryan K, Fields KW, et al. 2014. Opportunities for lean stability strategies. *Journal of Pharmaceutical Innovation* 9; 259–271.
72 White, E. 2009. Risk management for aseptic processing. *Journal of Validation Technology* Spring 2009; 25–33.
73 FDA, 2004. Pharmaceutical CGMPs for the 21st century- A risk based approach. Final Report. http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/Manufacturing/QuestionsandAnswersonCurrentGoodManufacturingPracticescGMPforDrugs/UCM176374.pdf. Accessed on July 20, 2015.

22

ICH Q10 Quality Systems

ICH Q10 Implementation at Genentech/Roche

Larry Wigman and Danny Ooi

Genentech, a Member of the Roche Group, South San Francisco, CA, USA

22.1 Introduction

ICH Q10 [1] builds on the fundamental concepts of good manufacturing practices (GMP), good documentation practices (GDP), and phase development by incorporating these concepts into a comprehensive quality management system described as pharmaceutical quality system (PQS). It is intended to harmonize various quality systems across different product life cycle stages with a focus on an integrated approach to quality risk management (QRM) and science. The guidance goes beyond the typical tactical quality guidance by recognizing that CMC is a continuum in product life cycle from research to development, to clinical trials, and further to technology transfer and commercial manufacturing, post-market pharmacovigilance and line extension, and eventual product discontinuation. Continuous improvements are encouraged rather than a philosophy that once a product is registered, changes are forbidden. The guidance recognizes that pharmaceuticals are a business with the responsibilities of management clearly outlined. Business-related tools often reserved for MBAs such as knowledge management (KM), design space (ICH Q8 [2]), and risk-based decision making (ICH Q9 [3]) are described. The main objectives of this guidance include product realization with appropriate quality attributes, establishing and maintaining a state of control, and facilitating continual improvement. While ICH Q10 is comprehensive in nature and based on established, standardized quality concepts (i.e., ISO quality management systems [4–6]) that cover the entire product life cycle, it is not intended to supersede GMP requirements (see Figure 22.1: the diagram in ICH Q10 Annex 2). In fact, as clearly indicated in ICH Q10, elements in the PQS that are beyond what is covered by regional GMP are considered optional.

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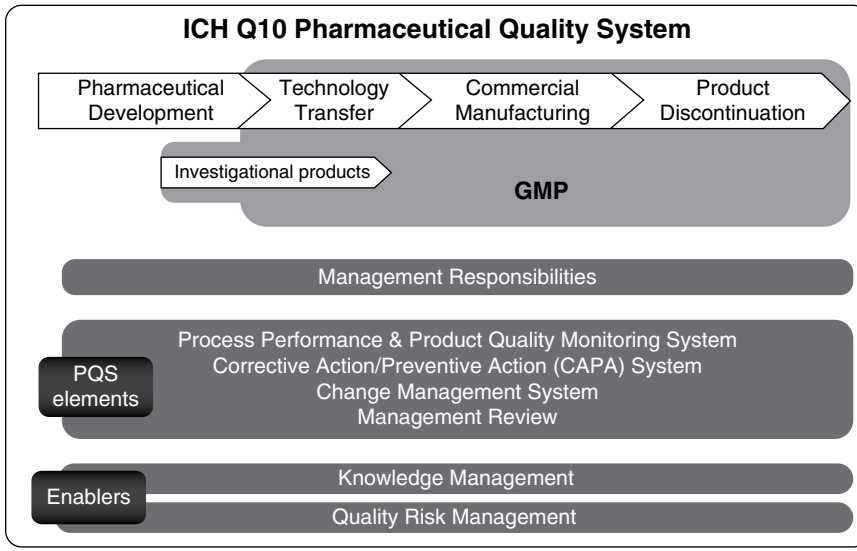


Figure 22.1 Diagram of the ICH Q10 pharmaceutical quality system model.

Building a strategy for a globally diverse company was a major undertaking and of the highest priority at Genentech/Roche. The company is highly diverse with existing products and pipelines including biomolecule therapeutics, small molecule pharmaceuticals, and antibody drug conjugates and involving every form of delivery from oral, parenteral, ocular, and inhalation. The operations include everything from discovery, research, development, clinical trial supply, commercial manufacturing, and global distribution for both homegrown and in-licensed products. Manufacturing includes both internal capacity and contract manufacturing organization (CMO) capacity at numerous locations across the globe. Indications cover every therapeutic area and treat diseases that affect major patient populations to small orphan disease populations. The one unifying concept was focusing on the patient and bringing them products with uncompromised quality.

The management vision for a PQS was to build a single comprehensive system of policies that covered the entire CMC continuum from preclinical to commercial and post-marketing. A multifunctional team of quality and technical representative was assembled under the direction of a high-level director. The approach involved site benchmarking, process mapping, recognizing stakeholders, and many hours of meetings to write this unified system of policies and guidances. The initial round was followed by a phased rollout of site implementation and continuous improvement to cover the multitude of process and operations involved. The following chapter will describe the evolution of the system from a strategic concept to a functional practice working on a

global level with examples primarily derived from a small molecule clinical phase development group.

22.2 PQS Considerations

Designing an ICH Q10 compliant quality system involves the following design and content considerations abstracted from the guidance:

- Design, organization, and documentation of the PQS should be well structured and clear to facilitate common understanding and consistent application.
- Elements of ICH Q10 should be applied in a manner that is appropriate and proportionate to each of the product life cycle stages, recognizing the different goals and knowledge available for each stage.
- The size and complexity of the company's activities should be taken into consideration when developing a new PQS or modifying an existing one. The design of the PQS should incorporate appropriate risk management principles. While some aspects of the PQS can be company-wide and others site specific, the effectiveness of the PQS is normally demonstrated at the site level.
- The PQS should include appropriate processes, resources, and responsibilities to provide assurance of the quality of outsourced activities and purchased materials.
- Management responsibilities should be identified within the PQS.
- The PQS should include the following elements: process performance and product quality monitoring, corrective and preventive action (CAPA), change management, and management review.
- Performance indicators should be identified and used to monitor the effectiveness of processes within the PQS.

As a continuously operating company founded in 1896, Roche had developed a complex legacy of quality management systems and policies. The acquisition of Genentech and integration of these legacy systems further complicated the quality environment. Genentech/Roche management recognized the importance of ICH Q10, and in 2009 the quality management team set out to build one global PQS. The logic driving the decision to unify a multitude of operations under one system included the following:

- Health authorities, patients, and stakeholders view the company as a single entity.
- Following the legal requirements for GMP in this regulated industry ensures the company's right to operate; clear quality requirements (QRs) reduce risk to the patient, set uniform standards for executing GMP, and ensure management involvement.

- Enables agility when making changes, ensures continual improvement, and facilitates transfer of processes, products, and people.
- Ensures that the company has agreement on the interpretation of phase-appropriate GMP requirements.

Six key areas were tackled each with expert leadership and teams composed of major stakeholders:

1) PQS documents
2) Change management*
3) CAPA*
4) Discrepancy management*
5) QRM
6) Governance, process mapping, and KM

*Common IT application—TrackWise® [7]

The commitment of resource was significant as anticipated to achieve the broad scope of the initiative. A multiyear development and phased rollout was planned over the course of 4 years along with an optimization and refinement phase of 2 years. The PQS is under constant continuous improvement and embraces the spirit of the guidance in this respect.

22.3 PQS Documents

Developing a comprehensive collection of documents covering diverse operations from preclinical to post-marketing was a major undertaking. To begin the process, a new system was developed to standardize the review process, manage the review cycles, and reconcile comments. Subject matter experts (SMEs) were nominated from each of the stakeholder functional areas to collaborate on the creation of one harmonized GMP PQS to describe the QRs and global standard processes (SOPs) for the combined organization. Priorities for the implementation focused on harmonization, ease of implementation (considering resources and budget), and compliance gaps.

The process was mapped into five key steps as shown in Figure 22.2.

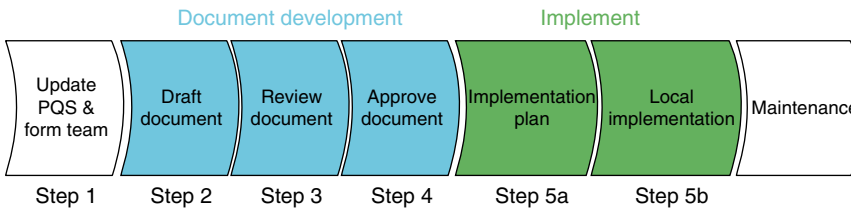


Figure 22.2 PQS document implementation map.

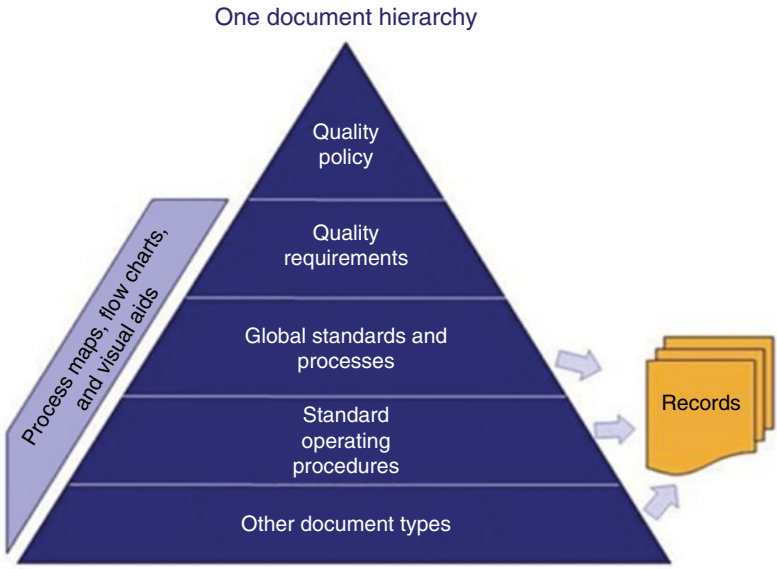


Figure 22.3 Document hierarchy.

As described in ICH Q10, the documents were organized into four major categories including management responsibilities, system elements, operations, and enablers. A single document hierarchy was developed to cover the range of policies, QRs, and procedures as illustrated in Figure 22.3.

To aid in building a comprehensive system of documents, detailed process maps were built, and the necessary changes in documentation were captured in the TrackWise change control system as records. Thus, progress could be well documented and continuous improvement made.

22.3.1 High-Level Documents

Strategically documents were written and implemented from the top down to set the vision, define management oversight, and establish the writing process. The first wave of implemented documents covered some of the new documents described in ICH Q10 such as a quality policy and a quality manual as well as critical areas with global importance. These first-wave documents are listed as follows:

- Quality Policy (POL)
- Quality Manual (QR)
- Management Review (GSP)
- Quality and Compliance Governance, Roles, and Responsibilities (GSP)
- Regulatory Inspection Management (QR)

- Regulatory Compliance Process (QR)
- Product Release (QR)
- Development and Deployment of Global Documents (GSP)

POL (Quality Policy), QR (Quality Requirement), GSP (Global Standard Operating Procedure)

22.3.2 Local Implementation

Local functional groups were charged with implementing the remaining applicable documents. For example, approximately 115 QRs and GSPs were applicable to the research and development organization. Since the goal was to build a single set of global documents for a diverse company covering all products from biomolecule therapeutics to small molecule pharmaceuticals and all operations from discovery to commercial manufacturing for both internal capacity and CMO capacity, local implementation uncovered many challenges.

To accurately capture the resource and compliance issues involved with implementing new global documents, a "local implementation assessment" was performed for each applicable document. The assessment was tracked through TrackWise®. Key knock-on effects to current local policies and key issues needing remediation were also captured in a systematic way. A representative assessment form illustrates the Genentech/Roche approach as contained in Table 22.1.

Successful local implementation requires continuous improvement with much discussion centered on risk-based assessments required for phase-appropriate GMPs and clarifying document interpretation. More detailed procedures and business processes were needed to ensure alignment and implementation. A hierarchy of documents from strategic and global to detailed local SOPs along with continuous improvement was the ultimate successful outcome.

22.4 Innovation and Continual Improvement

In ICH Q10 the goals of a quality product and a well-controlled manufacturing process are not static. Maintaining quality and controlled manufacturing throughout the product's lifecycle require continual improvements. Routine quality monitoring and investigations are an expectation. Data from quality monitoring and investigations can lead to necessary CAPAs focused on quality risk to patients as described in the following excerpt:

> The pharmaceutical company should have a system for implementing corrective actions and preventive actions resulting from the investigation of complaints, product rejections, non-conformances, recalls,

The following local actions will be taken (including site documents to be updated/retired and training to be completed) in order to complete the implementation of **POL**/**QR**/**GSP** # :

#	Elements of QR/GSP that need local implementation	Current situation that needs remedy (e.g. Gaps)	Description of activity to be completed (corrective action)	Describe how activity (proof of efficacy) will be documented	Responsible person	*Due date/ effective date	Resource requirements
1							
2							

Local document impact:

As part of determining local impact, the following table lists local or departmental procedures that must be revised or retired to complete implementation of new PQS document.

Document name and ref #	Retire or revise?	Responsible person	Target approval/effective date

During the implementation phase, sites/functions must identify which documents remain as the "official" documents to follow until the new PQS document can complete the implementation phase:

Official document ref # and name (list local/functional area procedures)	Target date to retire/revise and adapt to new PQS document

Table 22.1 GSP document implementation.

deviations, audits, regulatory inspections and findings, and trends from process performance and product quality monitoring. A structured approach to the investigation process should be used with the objective of determining the root cause. The level of effort, formality, and documentation of the investigation should be commensurate with the level of risk, in line with ICH Q9 <Quality Risk Management>. CAPA methodology should result in product and process improvements and enhanced product and process understanding.

In a diverse organization covering preclinical to post-marketing products, change is an expectation, and protecting the patient with phase-appropriate risk-based decisions is critical. In the early stages of development, the rate of change is expected to be rapid and data driven with appropriate documentation as well as management review. Genentech/Roche has developed a phase-appropriate risk-based approach with ICH Q10 compliance in mind. The use of a common system (TrackWise®) for change management, discrepancy management, change control, CAPA with built-in quality oversight, regulatory oversight, and management approval has been implemented. The nesting of policies covering development from preclinical to commercial phases with appropriate differentiation for development and commercial policy is shown Figure 22.4.

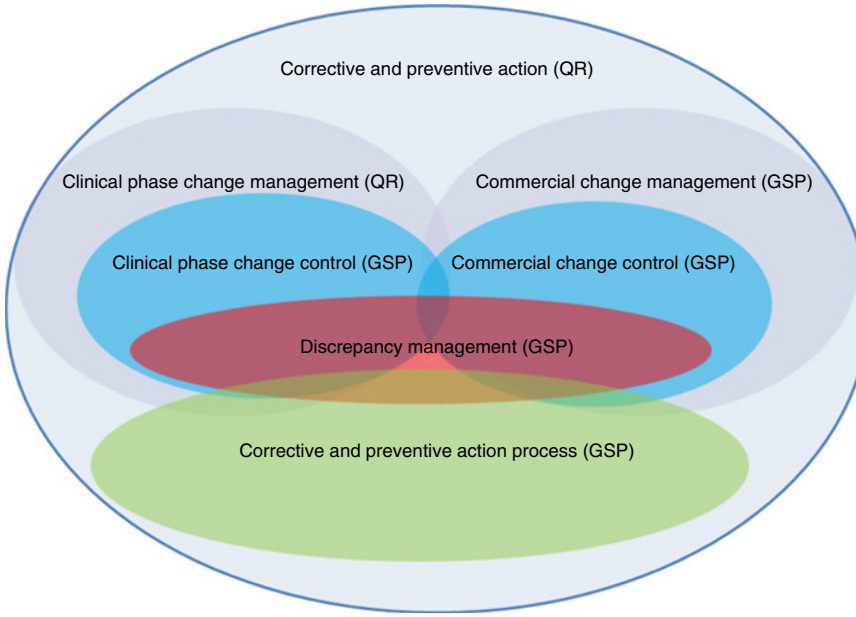

Corrective and preventive action (QR)
Clinical phase change management (QR)
Commercial change management (GSP)
Clinical phase change control (GSP)
Commercial change control (GSP)
Discrepancy management (GSP)
Corrective and preventive action process (GSP)

Figure 22.4 Innovation and continual improvement document nesting.

The process covers "end-to-end" development from preclinical to commercial in four phases as illustrated in Figure 22.5:

- In phase 1 the quality strategy is being planned. Systems and processes to be monitored are identified along with the appropriate testing and measurements. For critical processes/systems management, review should be performed to address quality and regulatory requirements.
- In phase 2 measurement and data analysis is initiated. These data are used to identify potential CAPAs or investigations.
- In phase 3 the CAPA process is used to advance changes that improve quality and potentially mitigate issues and investigations.
- In phase 4 management is briefed and management reviews conducted.

22.4.1 Change Management and Change Control in Clinical Development

During clinical development changes related to API process and scale, drug product process, composition, scale, shelf life, strength, and appearance are to be expected as a new drug is advanced toward commercialization. The principle behind change management in clinical development is to ensure that changes do not adversely impact product quality, safety, and efficacy via risk assessments and QRM. Change management objectives during this phase are focused on documenting the changes during clinical development, ensuring that changes are appropriately assessed for quality, manufacturing site (including CMOs), and regulatory impact and that the changes are endorsed by the technical development team SMEs. This process triggers the appropriate regulatory submissions/amendments and updates to clinical trial materials/labeling, thus ensuring GMP and regulatory compliance of clinical supplies. Figure 22.6 documents the flow and scope of change management in the clinical phase of development.

The principle behind change control in clinical development is to ensure that changes are properly assessed, approved, and documented as a gate to implementation. By using a risk-based approach, three levels of impact can be assigned to each change with each level triggering the appropriate assessments and required approvals. The assessments and approvals must be made by trained staff with current credentials:

- Level 1 changes are low risk changes that have no product quality impact, no regulatory impact, and no manufacturing site impact and do not require additional validation. These changes require only quality approval and can be implemented without validation or verification testing. Some examples of level 1 changes include editorial and formatting changes to documents, minor changes in analytical methods such as mobile phase gradient with no impact on validation or regulatory dossiers, and like-for-like changes in instruments.

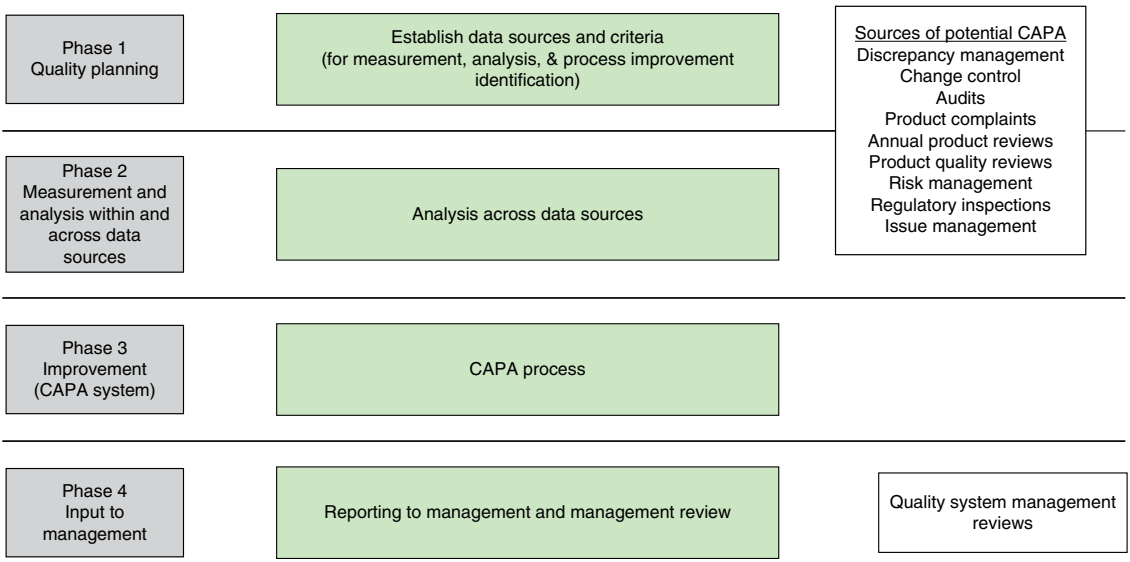


Figure 22.5 End-to-end development.

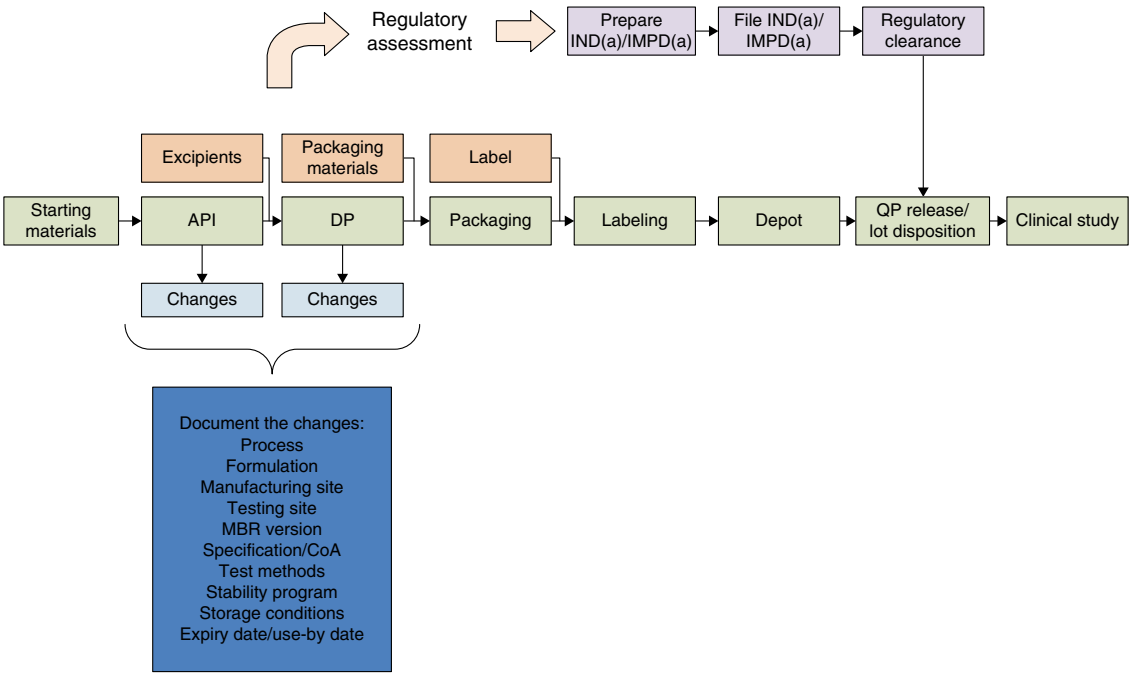


Figure 22.6 Flow diagram of change management for clinical development.

- Level 2 changes may impact product quality or validated systems; however, these changes have no regulatory impact and do not impact existing quality agreements. These changes require assessment by an SME and often additional data to facilitate the assessment. Multiple approvals may be needed. Some examples of level 2 changes include changes to master batch records, supplier changes for starting materials or excipients, and changes to analytical methods requiring validation, but not dossier amendment.
- Level 3 changes are considered to be substantial with impact to product quality and/or impact to regulatory dossiers and/or impact to quality agreements along with additional validation/qualification. These changes require multiple assessments and approvals. Some examples of level 3 changes include change in API synthesis route, critical process changes, new manufacturing sites, and changes in analytical methods or specifications requiring dossier amendments.

22.4.2 Discrepancy Management

Discrepancies can be defined as unplanned, unexpected, or atypical events in a GMP operation. The discrepancy management process includes the detection, classification, assessment, remediation, and closure of these events. A single policy is applicable to all GMP activities globally, including, but not limited to, batch production processes, computer systems, utilities, equipment, raw materials, environmental conditions, and QC testing. A single electronic system, TrackWise®, is used to document and assess discrepancies. Critical types of discrepancies are described in separate policies as shown in the Figure 22.7.

Discrepancy management is a risk-based process. Very simple discrepancies with clearly assignable cause and no impact to product quality, patient safety, validation, or regulatory dossiers can be closed by simple documentation as process comments in batch records or laboratory notebooks without TrackWise® records. More significant discrepancies are escalated. Some examples of simple discrepancies closed as a process comment include the following:

- A lab error that invalidates the test session and/or result with additional samples available for testing.
- A sample is compromised and additional sample is available for testing.
- System suitability acceptance criteria are not met due to inadequate column conditioning.
- The flow diagram for preliminary assessment of discrepancies is shown in Figure 22.8.

Discrepancies are escalated to deviations due to a departure from GMP that requires documentation of impact, cause, additional actions, and technical assessment. These deviations are time sensitive and must be initiated within one business day and closed within 30 business days. Discrepancies are further escalated to investigations when the event has potential product quality or patient

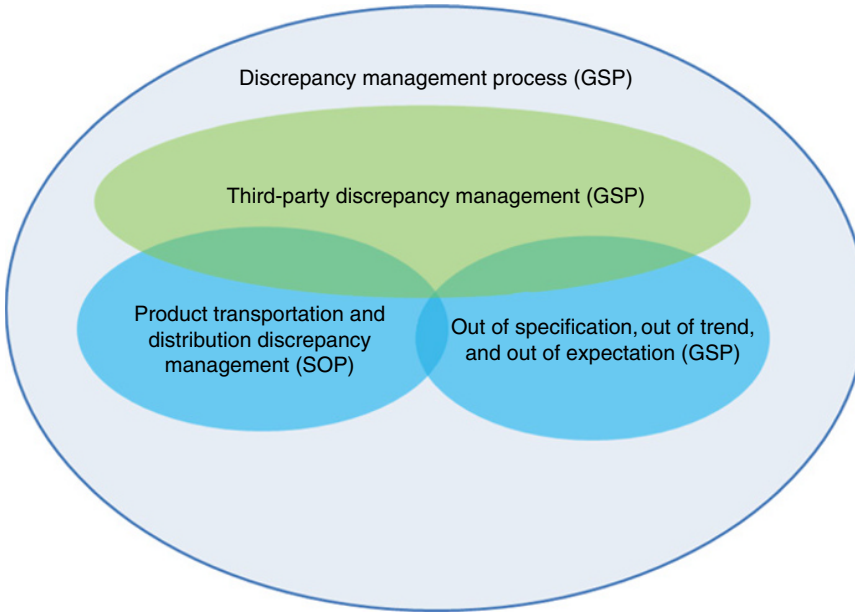

Discrepancy management process (GSP)
Third-party discrepancy management (GSP)
Product transportation and distribution discrepancy management (SOP)
Out of specification, out of trend, and out of expectation (GSP)

Figure 22.7 Discrepancy policy documents nesting.

safety risk or potential for impact to other sites, requires extensive testing or technical assessment to understand cause and impact, and may require remediation action. The deviation management process flow is illustrated in Figure 22.9.

Investigations are escalated to discussion at a quality review board (QRB). The QRB is an assembly of senior-level individuals from operations and quality whose purpose is to provide guidance on the strategy for resolution of these investigations. The QRB will confirm the investigation classification and endorse the investigation strategy and scope. This review process provides quality management with an understanding and context of quality issues escalated to investigation and the potential for multi-site impact. The QRB ensures quality issues are appropriately escalated. Escalation may include field action management and health authority reporting, if needed. The QRB will endorse final investigation conclusions and proposed actions including CAPAs.

22.5 Auditing, CAPAs, and Management Oversight

Auditing and inspections are a critical part of continuous improvement and GMP compliance monitoring. A good auditing system includes management review and oversight, leading to a culture of risk-based decision making and meeting the ICH Q10 goals of product realization with appropriate quality

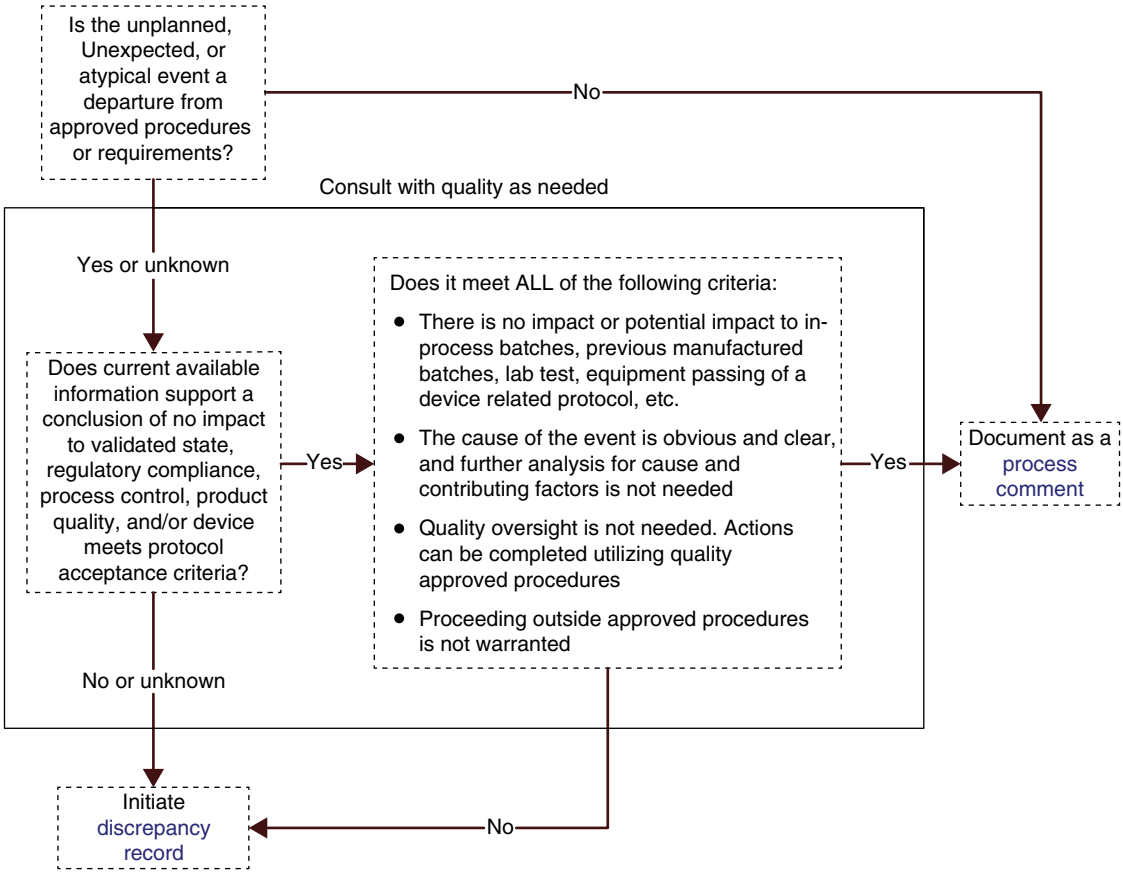

Is the unplanned, Unexpected, or atypical event a departure from approved procedures or requirements?
No
Consult with quality as needed
Yes or unknown
Does current available information support a conclusion of no impact to validated state, regulatory compliance, process control, product quality, and/or device meets protocol acceptance criteria?
Yes
Does it meet ALL of the following criteria:
There is no impact or potential impact to in-process batches, previous manufactured batches, lab test, equipment passing of a device related protocol, etc.
The cause of the event is obvious and clear, and further analysis for cause and contributing factors is not needed
Quality oversight is not needed. Actions can be completed utilizing quality approved procedures
Proceeding outside approved procedures is not warranted
Yes
Document as a process comment
No or unknown
Initiate discrepancy record
No

Figure 22.8 Preliminary assessment of discrepancies.

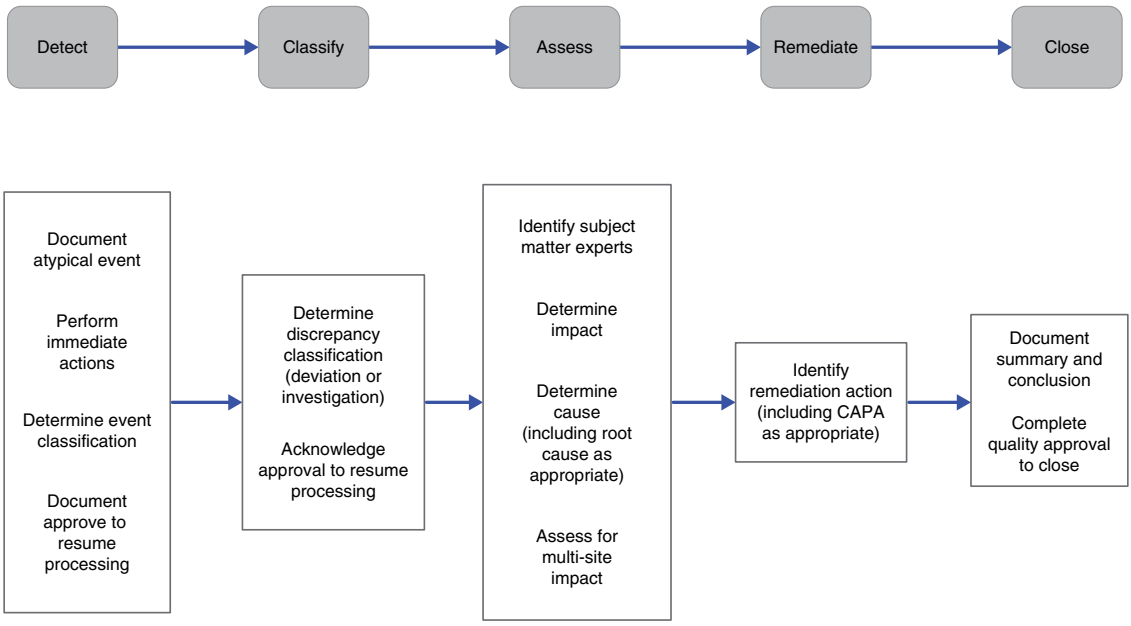


Figure 22.9 Deviation management process flow.

attributes, establishing and maintaining a state of control, and facilitating continual improvement. Auditing and inspection are expected by health authorities as exemplified in the FDA Inspection Manual [8] and the EudraLex self-inspection requirements [9]. Audits are designed to evaluate current practices against the following key criteria:

- Regulatory health authorities' GMP requirements and expectations
- PQS compliance and harmonization
- Current industry practices as defined and described in guidance documents or ISO standards
- Compliance with regulatory submission dossiers for commercial products or equivalent for investigational medicinal products
- For outsourced activities, compliance with the quality agreement and ability to produce material according to approved technical agreements and supplier specifications

Auditing and self-inspections should occur regularly (at least annually) and include detailed reports, management review, and time-bound CAPAs. Assessment of CAPAs should be done using a risk-based approach such that critical findings are addressed rapidly. Audit findings can be classified as "critical, major, or minor" based on public health, product and/or data integrity risk, or the extent of a system's failure. Detailed reports with classified findings identified during audits should be distributed to the auditee, impacted departments, site management and the corresponding inspection team quality unit head, and if applicable EU QP or responsible person and the head of quality of the impacted operational unit. The overall ability of a site to operate satisfactorily should be evaluated. Results from self-inspections or internal audits do not need to be shared with outsider auditors/inspectors including health authorities. Inspections should focus on key GMP areas and elements as described in Table 22.2.

Management oversight and responsibilities are an important part of ICH Q10 compliance. After establishment of a PQS and a culture of continual improvement management, oversight is a critical part of the audit, inspection, and regulatory review system. Management oversight and responsibilities are an important part of ICHQ10 compliance. After establishment of a PQS system and a culture of continual improvement management oversight is a critical part of the audit, inspection and regulatory review system. Management oversight is the key to:

- Providing appropriate resources throughout the life cycle of a site/product;
- Ensuring the appropriate sites and functions are involved in PQS Development/Harmonization and the response process for any findings that apply across sites;
- Reviewing audit reports;
- Reviewing, evaluating and approving audit responses including CAPA criticality and timelines for completion.

Table 22.2 Inspection focus areas and elements.

Area	Example elements that may be included
Quality system	Review and approval processes
	Discrepancy management
	Change control
	CAPA
	Complaints
	Recalls
	Personnel aspects (e.g., job descriptions, training, contractor management, etc.)
	Design control
Facilities, utilities, equipment	Contamination control
	Cleaning
	Equipment qualification
	Critical utilities (water systems, air systems)
	Environmental monitoring
	Maintenance and calibrations, sanitation, pest control, etc.
Production system	Process validation
	Production control
	Production records and adherence to specifications
Materials management	Testing and release
	Supplier qualification and changes
	Computerized processes
	Qualification of storage areas
	Shipping and distribution
	Returns
Laboratory controls	Sampling practices
	Analytical method validation
	Discrepancies
	Raw data
	Stability program
	Management of reference and reagent materials
Packaging and labeling	Reconciliation and storage of labels
	Label issuance
	Label control and verification
	Artworks

The management review system should contain the elements as abstracted from ICH Q10:

a) The management review system should include:
   1) The results of regulatory inspections and findings, audits and other assessments, and commitments made to regulatory authorities
   2) Periodic quality reviews, which can include:
      i) Measures of customer satisfaction such as product quality complaints and recalls
      ii) Conclusions of process performance and product quality monitoring
      iii) The effectiveness of process and product changes including those arising from CAPAs
   3) Any follow-up actions from previous management reviews:
b) The management review system should identify appropriate actions, such as:
   1) Improvements to manufacturing processes and products
   2) Provision, training, and/or realignment of resources
   3) Capture and dissemination of knowledge

22.6 PQS Enablers

As indicated in ICH Q10, QRM and KM are the two enablers in the implementation of PQS that result in accomplishing three main objectives: support "the PQS goal of achieving product realization, establishing and maintaining a state of control, and facilitating continual improvement." These two enablers span the entire product life cycle.

22.6.1 Quality Risk Management

QRM is a systematic method of organizing information to support a risk management decision as more thoroughly described in ICH Q9. It is a systematic process for the identification, assessment, control, communication, and review of risks to the quality of the drug (medicinal) product across the product life cycle. As part of the foundation of quality by design (QbD), QRM guides the development activities and monitors the progress of the development efforts through commercialization and product discontinuation. Being one of the two PQS enablers, QRM is defined in ICH Q10 as "… integral to an effective pharmaceutical quality system. It can provide a proactive approach to identifying, scientifically evaluating and controlling potential risks to quality. It facilitates continual improvement of process performance and product quality throughout the product lifecycle."

In Genentech/Roche, a GSP was developed specifically for QRM process and global operational instructions, which form the basis of conducting quality risk assessment (QRA), establishing risk controls, reviewing risks, and communicating QRM activities. The principles and examples of tools provided in ICH Q9 are some of the key elements in this GSP. The scope of this GSP covers sites

involved in development through product divestment activities, product supply management, material and quality services, regulatory functions, and investigational medicinal product and commercial products. The goal is to integrate the QRM into the relevant elements of PQS and quality processes, such as product/process development and life cycle, quality auditing, design controls, change management, discrepancy management, CAPA, etc.

A typical QRM process consists of the following six steps, which may involve QRM lead and sponsor, facilitator, SMEs, and quality unit:

1) *Initiation of QRM process*—Planning and documentation are adequately implemented to enable robust risk assessment process, including later steps (e.g., risk review).
2) *Risk assessment*—Appropriate risk assessment methodologies are employed according to the problem description, risk assessment objectives, phase appropriateness, and other relevant factors.
3) *Risk control*—The decisions of risk reductions and/or risk acceptance are made by the decision makers that include quality, consideration of all relevant factors (i.e., totality of circumstances), and prioritization (quantitative and/or qualitative) of risk control actions. See Figure 22.10 as an example of color-coded qualitative risk evaluation.
4) *QRM output/results*—The outcomes are summarized, documented, and approved by decision makers.
5) *Risk review*—Identified risks are reviewed and monitored (over a specified time interval) to determine if there is any impact to the original QRM decisions.
6) *Risk communication*—Sharing of risk information and risk management activities between the QRM team and other stakeholders who are individual/organizations that might benefit or be impacted by the risk management process.

The level of effort, formality, and documentation applied to the aforementioned QRM process is commensurate with the level of risks. The risk management documents are maintained throughout the life cycle of the associated product, process, or system and must be readily available.

An example of how these steps are incorporated into the QRM is provided in Figure 22.11.

		Probability of occurrence		
		Low	Medium	High
Severity	High	Medium risk	High risk	High risk
	Medium	Medium risk	Medium risk	High risk
	Low	Low risk	Low risk	Medium risk

Figure 22.10 An example of qualitative risk evaluation.

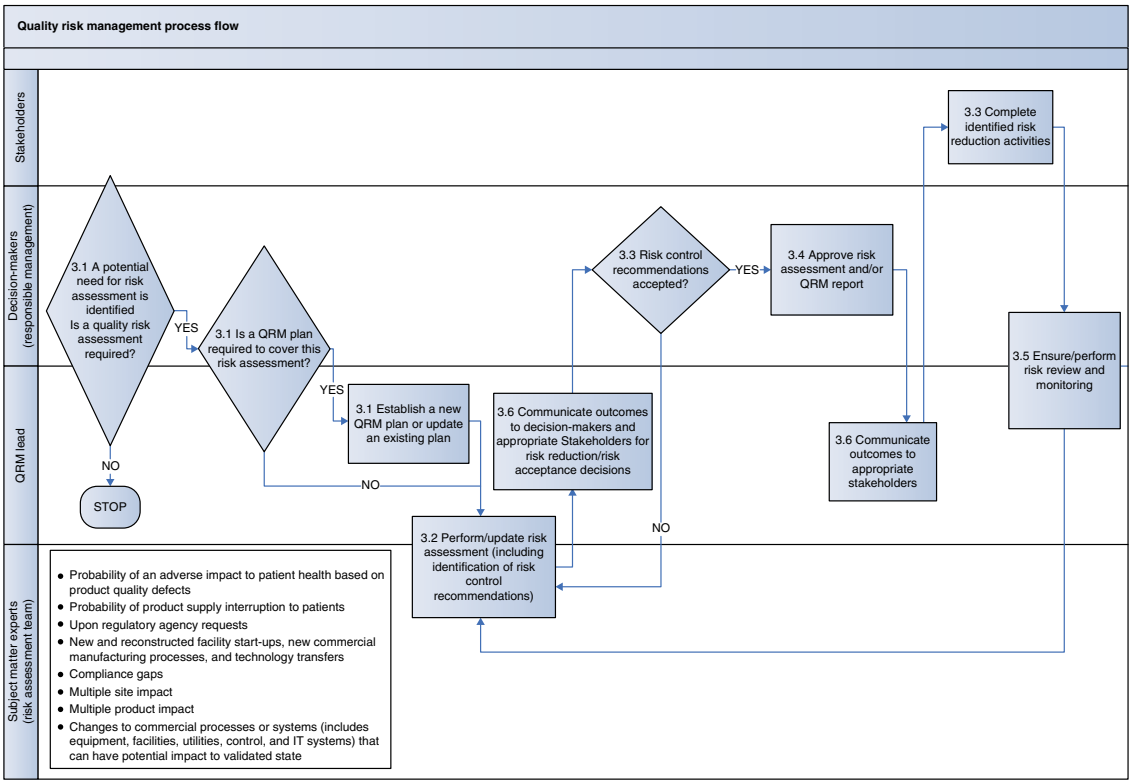

Quality risk management process flow
Stakeholders
Decision-makers (responsible management)
QRM lead
Subject matter experts (risk assessment team)
3.1 A potential need for risk assessment is identified Is a quality risk assessment required?
YES
3.1 Is a QRM plan required to cover this risk assessment?
YES
3.1 Establish a new QRM plan or update an existing plan
NO
NO
STOP
3.6 Communicate outcomes to decision-makers and appropriate Stakeholders for risk reduction/risk acceptance decisions
3.3 Risk control recommendations accepted?
YES
3.4 Approve risk assessment and/or QRM report
3.3 Complete identified risk reduction activities
3.5 Ensure/perform risk review and monitoring
3.6 Communicate outcomes to appropriate stakeholders
NO
3.2 Perform/update risk assessment (including identification of risk control recommendations)
Probability of an adverse impact to patient health based on product quality defects
Probability of product supply interruption to patients
Upon regulatory agency requests
New and reconstructed facility start-ups, new commercial manufacturing processes, and technology transfers
Compliance gaps
Multiple site impact
Multiple product impact
Changes to commercial processes or systems (includes equipment, facilities, utilities, control, and IT systems) that can have potential impact to validated state

Figure 22.11 Quality risk management process flow.

22.6.2 QRM in Product Development

Built on the aforementioned QRM framework, various QRA execution points were placed throughout the product development from small to commercial production scales. Such strategic use of QRAs is a critical part of the implementation of Roche QbD principles. Shown in Figure 22.12 is an example of how QRM is implemented in the pharmaceutical development of small molecule. Note that the methodology (e.g., analysis of severity, probability, etc.)

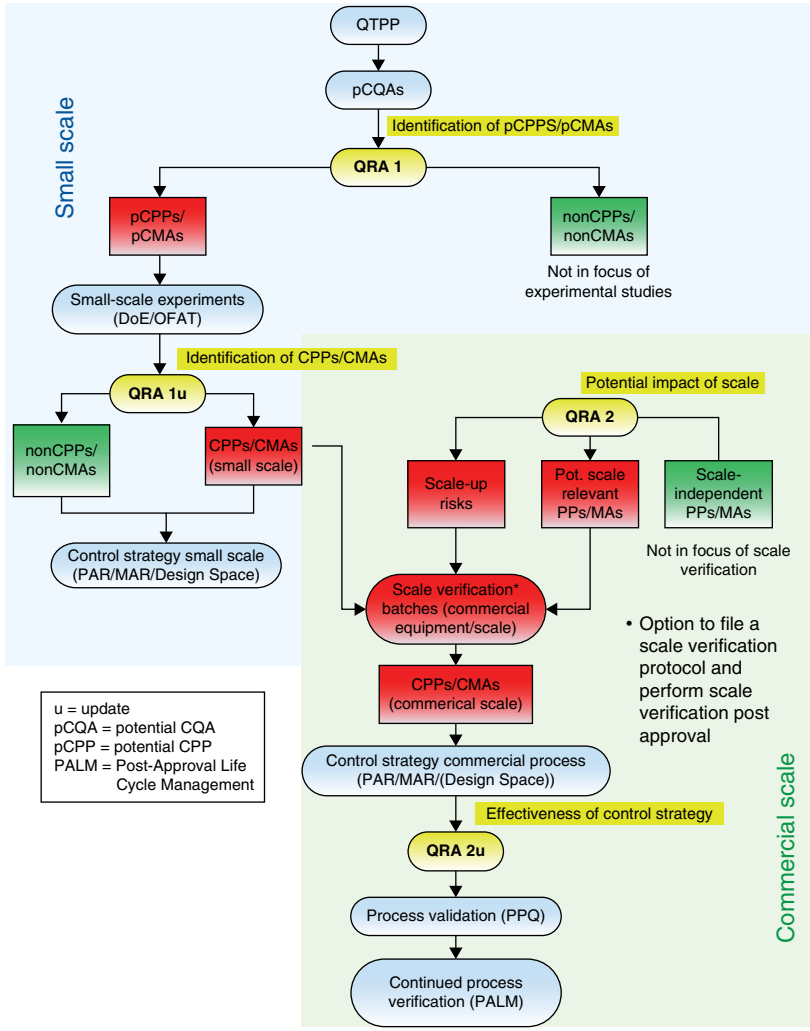


Figure 22.12 The utilization of four QRAs at different stages of pharmaceutical development of small molecule.

Drug product pCQA's	Material attributes		Process steps			
	API	Excipients	Granulation and drying	Final blend	Tableting	Coating
Appearance	Low	Low	Low	Low	Low	High
Content/assay	Low	Low	High	Low	Low	Low
Uniformity of dosage units	Low	Low	Low	Low	Low	Low
Dissolution	High	Low	High	Low	Low	Low
Degradation products	Low	Low	Low	Low	Low	Low
Microbiology	Low	Low	Low	Low	Low	Low
Identity	Low	Low	Low	Low	Low	Low

Figure 22.13 An example of the summary of QRA2 used to identify the CMAs and CPPs that impact drug product pCQAs.

used in QRAs is different from the QRMs; the differences are intentional to better support a QbD-based process development. A multidisciplinary team, which includes experienced members from technical and regulatory functions, is assembled to perform the QRA.

Each of the four QRA points is implemented at different development phases and serves to guide the development activities and monitor their progress:

1) *QRA1*—Identification of pCPPs and pCMAs based on prior knowledge, product characteristics, etc. These pCPPs and pCMAs could affect drug product quality attributes and process robustness. It is in small/pilot scale and typically occurs at the start of process development after the market formulation is defined. The conclusion of this assessment will guide the subsequent development activities.
2) *QRA1u*—Identification of CPPs and CMAs based on experiments; it typically initiates after small/pilot-scale development.
3) *QRA2*—Identification of scale-up risks and potential scale relevant pCPPs and pCMAs (e.g., scale, equipment type/design to site-specific processes, etc.); it is in commercial scale and occurs prior to transfer to commercial manufacturing. An example of the summary of QRA2 is provided Figure 22.13.

4) *QRA2u*—confirmation of the effectiveness of commercial control strategy; it is in commercial scale and occurs after scale verification and registration batches in commercial scale.

22.6.3 Knowledge Management

According to ICH Q10, KM is a systematic approach to acquiring, organizing, analyzing, storing, and disseminating information related to products, manufacturing processes, and components. Many of the concepts in ICH Q10 related to KM were derived from the medical devices field [10], and these concepts will be evident in this section. KM is one of the two key enablers of product and process life cycle within the Roche PQS. As defined in ICH Q10, both product and process knowledge created must be accessible throughout development and commercial manufacturing.

In order to effectively manage product and process knowledge, that is, enable use of prior knowledge and apply it to create new knowledge and capabilities, KM was developed to include four key capabilities:

1) Culture—Enables a culture of knowledge sharing and learning throughout the product and process life cycle and across organizational boundaries. KM increases the ability of individuals to access knowledge both explicit and tacit, to understand roles and responsibilities, and to be able to transfer knowledge to others.
2) Processes—Enables a standardized and harmonized knowledge flows from knowledge creation/collection and review/access to sharing and being used/reused.
3) Technology—Includes systems and technologies that enable information sharing across organizational boundaries.
4) Content—Refers to tacit and explicit knowledge, but also taxonomies, metadata, templates, and other supporting elements.

Central to these four capabilities is the product history file (PHF), which was developed to capture the knowledge of a product and its manufacturing process created during the product life cycle. The PHF provides an index of the PQS required documents for storage, retrieval, and applying product knowledge. Product knowledge comes from various sources that provide a detailed and comprehensive product and process history. The PHF centralizes the accumulated knowledge from pharmaceutical development to product discontinuation and encompasses analytical, product and process development, device development, and manufacturing experience as shown in Figure 22.14.

The main elements of the PHF are (i) the content used to capture product and process knowledge, (ii) taxonomy (i.e., classification schema) and

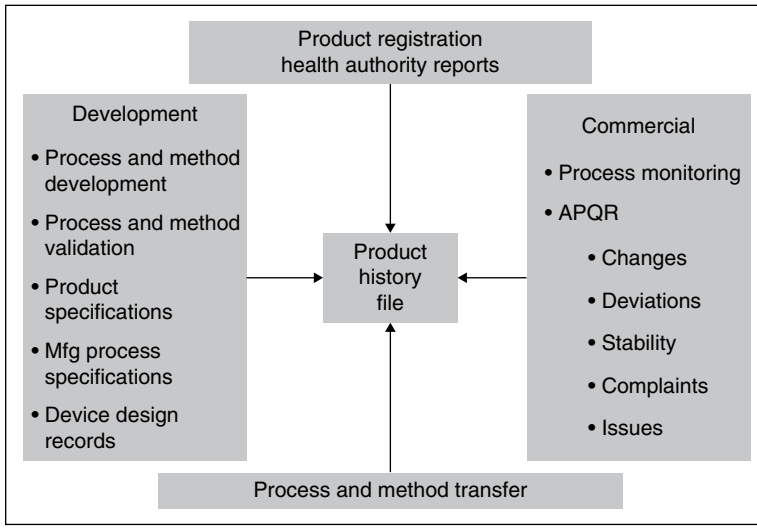


Figure 22.14 Components of the product history file.

metadata supporting searching and filtering, and (iii) an electronic repository to store the PHFs, which include deliverables from drug substance, drug product, packaging and packaging components, and, where appropriate, medical devices.

The PHF undergoes three life cycle phases:

1) *Knowledge creation*: Create/update product-specific PHF. Capture PQS required information into PHF from pharmaceutical development, technology transfer, and commercial manufacturing.
2) *Organization and communication*: The knowledge/information is organized and then stored in a transparent repository to provide global access. Knowledge dissemination is done through training programs and network meetings.
3) *Knowledge application*: The PHF is accessed and used to support improved problem solving, planning, and decision making, leading to continuous improvement and efficiencies. Knowledge is acquired throughout the product life cycle and updated and reviewed periodically. When a product is discontinued, the PHF is stored and retained according to appropriate retention policies and business practices.

Figure 22.15 shows the phases of the product and process KM life cycle and the KM capabilities.

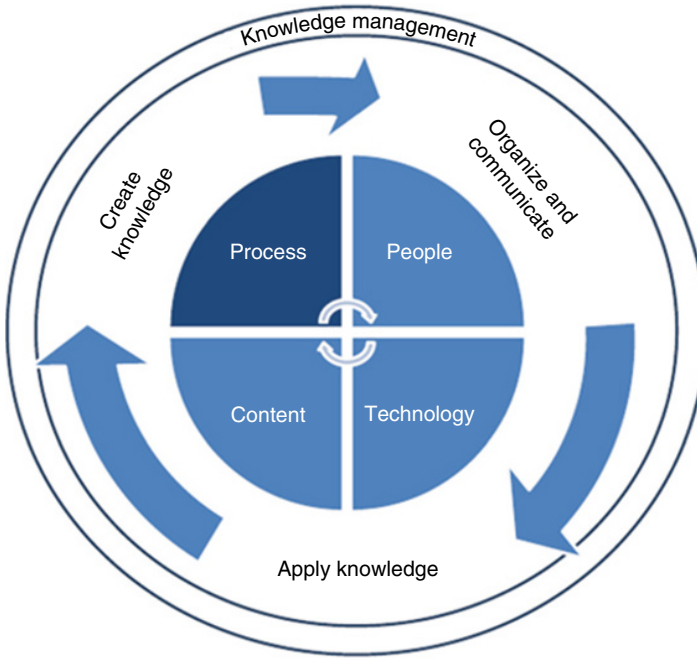


Figure 22.15 Product and process knowledge management program.

22.7 Benefits of ICH Q10 Implementation and Future Opportunities

The goal of ICH Q10 is to promote a philosophical and cultural shift away from discrete GMP compliance procedures for specific manufacturing sites and specific stages of a product life cycle to a comprehensive approach to address the continuum of a product life cycle. In this post-ICH Q10 environment, a harmonized PQS with enablers such as QRM and KM is leveraged to achieve the objectives of product realization with appropriate quality attributes, establishing and maintaining state of control, and facilitating continual improvement. The ICH Q10 Annex 1 describes the opportunities to enhance science- and risk-based regulatory approaches as shown in Table 22.3.

Major benefits realized from ICH Q10 implementation at Genentech/Roche were the development of a comprehensive documentation system covering diverse operations from preclinical to post-marketing, thus creating one harmonized GMP PQS. The harmonized PQS defined the QRs and SOPs for the

Table 22.3 Potential opportunities to enhance science- and risk-based regulatory approaches.

Scenario	Potential opportunity
1. Comply with GMPs	Compliance—status quo
2. Demonstrate effective pharmaceutical quality system, including effective use of quality risk management principles (e.g., ICH Q9 and ICH Q10)	Opportunity to: • Increase use of risk-based approaches for regulatory inspections
3. Demonstrate product and process understanding, including effective use of quality risk management principles (e.g., ICH Q8 and ICH Q9)	Opportunity to: • Facilitate science-based pharmaceutical quality assessment • Enable innovative approaches to process validation • Establish real-time release mechanisms
4. Demonstrate effective pharmaceutical quality system and product and process understanding, including the use of quality risk management principles (e.g., ICH Q8, ICH Q9, and ICH Q10)	Opportunity to: • Increase use of risk-based approaches for regulatory inspections • Facilitate science-based pharmaceutical quality assessment • Optimize science- and risk-based post-approval change processes to maximize benefits from innovation and continual improvement • Enable innovative approaches to process validation • Establish real-time release mechanisms

combined organization. The harmonized PQS established a mechanism for continuous improvements and change management to meet the needs of a diverse and growing company.

As the pharmaceutical industry moves ahead to meet unmet medical needs and make affordable treatments available to more patients, the cultural changes in ICH Q10 including continual improvement, science- and risk-based decisions, and adoption of new technological advances become increasingly important. Some of the key challenges to be overcome by PQS and the enablers of KM and QRM of interest to the authors include globalization of raw material sources, manufacturing, packaging, and supply chain; continuous processes and real-time/parametric release; novel therapies; and the evolving regulatory environment.

Acknowledgments

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References

1 ICH Q10, Pharmaceutical Quality System, June 2008.
2 ICH Q8(R2), Pharmaceutical Development, August 2009.
3 ICH Q9, Quality Risk Management, November 2005.
4 ISO 9000: "Quality Management Systems"—fundamentals and vocabulary.
5 ISO 9001: 2000: "Quality Management Systems"—requirements.
6 ISO 9004: "Quality Management Systems"—guidelines for performance improvements.
7 Trackwise®, a registered trademark of Sparta Systems, Inc.
8 FDA Inspection Manual 7356.002.
9 Eudralex Volume 4 Chapter 9—Self Inspections.
10 ISO 13485: 2003 Medical devices—Quality management systems; Requirements for regulatory purposes.

23

ICH Q11

Development and Manufacture of Drug Substance

Ronald Ogilvie

Pfizer, Sandwich, UK

23.1 Introduction

The fourth of the famous triad of the "new wave" ICH guidance documents examining science- and risk-based development, the final Business Plan and Concept Paper for the Q11 guideline on Development and Manufacture of Drug Substances, was endorsed by the ICH Steering Committee in April 2008. In contrast, ICH Q8(R2) [1]—pharmaceutical product development—was initiated in 2004. Therefore it almost appeared to some that the Q11 guidance was an "afterthought." Given that the principles of quality by design and design space, and other concepts associated with a science and risk-based approach to development, had been defined in ICH Q8, there was a suggestion from some voices that the Q11 guidance might be very short, simply a statement to the effect that "The concepts and principles of ICH Q8, 9 and 10 for the science and risk-based development of drug products, risk assessment and quality system can also be applied for drug substance development and manufacture." Certainly, the desired outcome for many stakeholders was neatly expressed in this brief sentence that ICH Q11 "should facilitate the application of 'enhanced' development approaches to drug substance development." However it is very apparent from consideration of the business plan that in reality a more comprehensive guideline was sought.

The ICH Q11 Business Plan talked of the need to "harmonise the technical information on drug substance, (both chemical and biotechnological/biological) process development and manufacture that is included in section S.2 of the Common Technical Document." Tangible value was attached to the benefit of such harmonization for both industry and regulators, in terms of resources saved in the preparation and review of documentation for drug

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substance registration, particularly the need to prepare "different" content for different regions. The Business Plan also suggested that such harmonized guidance on drug substance content might lead to a reduction in the number of assessor queries on section S.2 and a reduction in the resources spent answering such queries.

It would be hard for anyone to argue against the logic of the Business Plan and the potential benefits that might be subsequently derived, but it is also clear from the plan that the development of the guidance, at a detailed level, was going to bring with it certain challenges that might make the task considerably more complex than the development of ICH Q8 [1] guidance for drug products. In seeking to address S.2 content expectations for both small molecule and biological drug substances, the expert working group (EWG) faced the significant challenge of defining guidance applicable across a highly diverse range of materials. Guidance not only broad in its scope but also detailed enough to guide development and manufacture of any one drug substance from within this wide spectrum of substances. A critical decision for the EWG to make therefore was if this breadth and depth could be accommodated in one text or if it would merit two separable sister texts, that is, one focused on "small" synthetic drug substances, the other on biologics. Even this would have proved difficult with new modalities such as oligonucleotides and antibody drug conjugates blurring the edges of the traditional categories. In the end, one joint guidance document was produced.

Significant challenges were therefore apparent even before one considered that the text would also need to provide guidance related to both "baseline/traditional" and "enhanced" development approaches.

Furthermore, the scope of the guidance (section S.2 of the Common Technical Document (CTD)) also widened the challenge for the Q11 EWG beyond that faced by the Q8 [2] (drug product) EWG. S.2 (unlike P.2) contains a number of important subsections that provide manufacturing (section S.2.2), control (sections S.2.3 and S.2.4), and validation (section S.2.5) content, as well as section S.2.6 that contains "development" information and maybe best compares with the P.2 content described in ICH Q8.

Thus, one can appreciate the considerable practical magnitude of the task facing the ICH Q11 EWG before one even considers the "paradigm-changing" content of ICH Q8 that provided science- and risk-based principles of development. The Concept Paper for the Q11 guideline also promised to harmonize the "scientific and technical principles relating to the description and justification of the development and manufacturing process of drug substances," and to "take into consideration and provide examples as appropriate, for describing the principles and concepts which are included in ICH guidelines Q8 [1], Quality Risk Management (Q9) [2] and Pharmaceutical Quality Systems (Q10) [3]." This, combined with the intent for the guideline to cover all categories of

drug substance, clearly takes the guidance away from the simple concept of a guideline that would provide the means to align the development of drug substance to the principles of ICH Q8/Q9/Q10.

Sensibly the EWG determined that principles common between drug substance and drug product would not be "redefined" by the Q11 EWG but that existing statements of "common principles" would be lifted in from Q8(R2). However, the greater scope of Q11, together with the existence of technical elements of development quite specific to the drug substance (e.g., selection of starting materials, impurity management) meant that the EWG's challenge was a significant one.

The Concept Paper listed the goals of the proposed Q11 guideline as follows:

- Harmonize and encourage the submission of the relevant documents regarding the manufacturing process and its justification
- Outline the science-based concepts relevant to the design of a robust manufacturing process that reliably delivers a quality drug substance
- Recommend approaches for demonstrating process and product understanding, where possible through example
- Facilitate the regulatory evaluation process for authorities
- Address the complexity of different manufacturing processes and products
- Accommodate variable approaches to development and corresponding information to be provided as described in ICH Q8 [1]
- Address enhanced approaches to manufacturing that can also create a basis for alternative approaches to control the quality of a product and for the application of innovative technologies for the manufacture of drug substances (e.g., continuous manufacture)
- Address systematic approaches to drug substance development, application of quality risk management, and concepts such as design space and control strategies (including real-time release) over the life cycle of the product

Despite the scale of the task, many most likely held the view that at least a significant portion of the "hard work" on the development of the necessary underlying principles had been done by the ICH Q8 EWG. This in turn may well have led to a prevailing view that the Q11 EWG would make far more rapid progress than had been possible with earlier guidelines. Indeed, that there might be no need for the Q8–10 implementation working group (IWG), who had provided a set of Q&A clarifications and a position paper further describing the major principles of Q8 to remain active. This IWG was disbanded before the conclusion of the work of the Q11 EWG. One is left to wonder the difference it might have made for this group to have remained in place and have been formally tasked with supporting the ICH Q11 EWG.

The next question to address in the development of Q11 was "what happy band of warriors would assemble to tackle the Q11 dragon?" The challenge was significant; those involved would need expertise in multiple areas:

- Small molecule and large, biological development
- Manufacturing and regulatory knowledge (multiple regions)

The Q11 EWG first met in June 2008, with the concept paper having stated that a Step 2 consensus draft of the guideline, would be available in "4Q 2009." In terms of ICH meetings, thus, a very small number of ICH meetings were initially envisaged as being needed to reach this stage. As highlighted, even a cursory review of the intended scope of objectives makes clear the actual scale of the challenge, hence it is perhaps not surprising that in the end the process took considerably longer. Step 4 [4] was reached in May 2012.

23.2 The Q11 Guidance: Its Genesis and Birth Right

Not so long ago, a guidance or monograph on "how to do product development" might have been resisted, and yet ICH Q8 [1] and Q11 [4] are entitled "Development and Manufacture of…." How therefore did a shift in thinking occur that would allow the acceptance of such guidelines? The genesis of these guidelines can perhaps be traced back to rather practical and logistical matters.

It had been clear that one of the greatest and most valuable harmonizations that might come from the ICH process might be the delivery of a harmonized registration submission document. Certainly, the work to harmonize the CTD (ICH M4) [5] and in particular the Module 3 Quality sections led to some interesting findings. The three regions, United States, Japan, and EU, did not have common expectations for what information was needed from an applicant. All three regions would probably have concurred that the focus of review was on the drug substance and product and their controls (hence the work done to produce guidances ICH Q6A [6] andQ6B [7]), but the depth of contextual information expected to be provided differed significantly. Notably, the EU had a guideline on "Development Pharmaceutics" [8] that asked for a scientific rationale of the product's suitability, and the EU wanted to see such supportive content maintained in the new framework of the CTD. The other regions accepted that this type of information would be part of the new submission format for the drug product, in P.2 Pharmaceutical Development, and would be balanced by a section providing drug substance development information (section S.2.6). However, what was clear was that the expectations for informational content of such sections were not harmonized, and some further guidance would be required. Although ICH M4 was issued with some "illustrative text" that seemed to indicate "sectional

content expectations," it was stated in the M4 text itself that the CTD M4 guidance provided for a common *format* and did not describe agreed common CONTENT expectations. In close parallel with these discussions, it is interesting to note that the FDA issued drafts of guidance related to drug substance [9] and drug product development [10]: these guidances were lengthy (the FDA Drug Product guidance [10] being 66 pages compared with the dozen or so pages of the EU Development Pharmaceutics guidance). How, one might wonder, would harmonization be effected from such regional guidance?

One answer would be to discuss and agree harmonization at the level of "principles," that is, simply define WHAT has to be shown by the applicant to the assessor to assure both the quality of the development work conducted and the appropriateness of the manufacturing process and controls applied to the product and to avoid setting out detailed expectations of HOW such work should be done.

One can almost imagine some of the discussions: different applicants can conduct development in different ways and yet still provide an approvable product; some applicants may choose to do "more'" work than others, based on a variety of factors relevant to the product or the company; some products might be more complex than others and have different development needs.

Based on such thinking organizations will likely face a dilemma, stick with traditional approaches to development for drug product (ICH Q8 [1])/drug substance (ICH Q11 [4]) or adopt an "enhanced" approach, closely linked to rational utilization of quality risk management principles (Q9).

One can maybe also hear the echo of discussions wondering what value could be gleaned from an applicant sharing enhanced understanding with a regulatory agency. Linked to this, perhaps a realization that for enhanced product knowledge from development to be of true lasting value across the life cycle of a product, it would have to be embedded in the manufacturing quality system (ICH Q10 [3]).

Thus, the pragmatic, tactical need for "gap-filling" guidance had settled in the fertile ground of "the desire for innovation" to blossom into a new strategic vision for pharmaceutical regulation that would be science- and risk-based, empowering, and better support manufacturing quality while, at the same time, focusing and even reducing resources needed by all parties.

Such flexibility would allow for both "traditional" and "enhanced" approaches to development (Q8 and 11), as well as allow a clear linkage between the design and development focus of the product based on a structured and rational utilization of quality risk management (Q9) [2].

This also raised several other important questions. These included, if, for example, Q11 supported both traditional and enhanced approaches from an applicant's perspective, what is the benefit to be gained from sharing enhanced understanding with a regulatory agency? What is the link between enhanced product knowledge gained during development and its value across the life

cycle of a product? In addition, how might such enhanced understanding be embedded within the overall manufacturing quality system (ICH Q10) [3]?

Such questions may be reflected in the overarching statements common to both ICH Q8 [1] and Q11 [4]:

- "A greater understanding of the drug substance and its manufacturing process can create the basis for more flexible regulatory approaches."
- "A company can use either a traditional approach or an enhanced approach to drug substance development, or a combination of both."
- "The degree of regulatory flexibility is generally predicated on the level of relevant scientific knowledge provided in the application for marketing authorisation."

A major challenge for any reviewer is how to assess submissions "equally" when the contents may lie on a spectrum of sharing either "traditional" or "enhanced" understanding. This begins to show the underlying strategic challenge for a guidance document like ICH Q11 [4]. That is, how to both enable innovation in industry while at the same time not freeing an applicant from the appropriate degree of regulatory oversight?

A classical systems approach to this type of problem is to provide a structured, customer-focused framework for work and decision-making, in which each design and development decision should be rational and targeted at delivering "what the patient needs" while at the same time providing an overview of quality criteria that need to be met. This is a classic "cause-and-effect" quality management approach where a product or process can be designed to deliver an output and the quality of the output can be measured to drive continued learning. However, neither industry nor regulators were very comfortable with the classical "measure outputs" approach to assure quality, as quality is expected be the "built in," especially for some critical requirements (e.g., biopharmaceutical equivalence or stability), which were not considered to be readily assured by "simple release testing" of a single product attribute.

Nevertheless, this strong sense of planned development based on meeting patient need through the delivery and assurance of designed product and product quality attributes is the essential backbone of the science-based approach to pharmaceutical drug substance development and manufacture. This approach is effectively predicated upon the belief that the developer of the drug substance will have an intimate understanding of the planned use of the drug substance, which may not always be the case, but can allow for more focused development when this link exists. Thus, one can conduct rationally designed development of a drug product when one has understood the patient needs that the product must fulfill and can conduct rationally focused development of a drug substance when one has understood the drug product, and its manufacturing process, and how aspects of the drug substance impinge on the quality, safety, and efficacy of the drug product. In this way, one can see how

ICH Q11 [4] drives the development needs of the drug substance from the drug product. Even this interrelationship is an oversimplification of the challenges associated with drug substance development and manufacture. In many instances physical attributes of the final drug substance are critical to the development and manufacture of the drug product. These physical attributes (e.g., solid-state form, particle size distribution, solubility, even stability) are generally established at the end of the manufacture of the drug substance. Consequently the earlier steps in the manufacture are likely to be less relevant to the critical attributes of the drug substance/drug product interface. However the manufacturing process of the drug substance as a whole is surely important to both the industrial manufacturer and the regulator, as the manufacturing process can determine what the potential RISKS to quality are, and how consistent the quality of drug substance may be. How such risks are identified, assessed in terms of criticality and their subsequent mitigation/control, therefore becomes a critical aspect of development. Linked to this is the ability to communicate this overall process and understanding to regulators.

23.3 Manufacturing Process and Development

23.3.1 General Principles: The Fundamental Expectations Associated with Drug Substance Development

ICH Q11 [4], like ICH Q8 [1], recognizes that approaches to development can vary from company to company and from product to product. Nevertheless, and importantly, the guideline establishes a set of "baseline" expectations for an applicant. Manufacturing process development should include, at a minimum, the following elements:

- Identification of critical quality attributes (CQAs[1]) associated with drug substance (so that those characteristics having an impact on the drug product can be studied and controlled)
- Definition of an appropriate manufacturing process (and process description)
- Definition of a control strategy to ensure process performance and drug substance quality

These "baseline" expectations best define the true regulatory requirement. Thus, every application must determine the drug substance CQAs, those linked to the safety and efficacy of the product and product's manufacturability, that

1 A CQA is defined within Q11 as "A physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality."

then forms the basis of the drug substance specification. Every applicant must provide a process description that defines HOW the drug substance is manufactured (of course, a variety of levels of detail for such a process description are possible). Finally, every application must detail a control strategy for the manufacture of the drug substance, which is NOT just the specification for the drug substance but also the controls established for input materials and in-process/isolated intermediate materials as well as, potentially, process parameters that are utilized to provide direct control over a CQA of the drug substance.

The fundamental responsibility of the manufacturer and regulators is to ensure that the drug substance that reaches the patient is of appropriate quality at its time of use, and thus the focus of regulation (and manufacturing compliance) is on implementing the control strategy and manufacture in accord with the approved process commitments described. However, it is important to note that, just as approaches to development can differ, the consequence of having different levels of product and process understanding from different approaches to development can be that the content of the process description and/or the control strategy can also differ from applicant to applicant and product to product, based on the degree of understanding that the applicant has of the product and process. ICH Q11 [4] acknowledges this in its introduction:

> In a traditional approach, set points and operating ranges for process parameters are defined and the drug substance control strategy is typically based on demonstration of process reproducibility and testing to meet established acceptance criteria. In an enhanced approach, risk management and scientific knowledge are used more extensively to identify and understand process parameters and unit operations that impact CQAs and develop appropriate control strategies….

What does this mean in practice? An applicant must list CQAs, a process description of all important parameters, and a control strategy for all CQAs of the drug substance. At one hypothetical end of the spectrum of investment in product and process understanding (the minimal end), an applicant will lack any specific knowledge to differentiate these aspects, and thus every potential quality attribute must be seen as critical. Similarly every possible detail of a process description may be critical resulting, theoretically at least, in the need to implement every possible control. Thus the minimal "landscape" for a submission is a highly detailed but limited submission where process parameter limits will be set exclusively based on experience and process capability (per ICH Q6A [6] and Q6B [7]). In many cases, especially in the case of accelerated development, manufacturing experience is likely to be very limited (small numbers of manufacturing campaigns/small number of actual batches), and hence process understanding derived entirely from manufacturing experience is very likely to be narrow, potentially leading to the imposition of tight process parameters

combined with extensive testing. Clearly such a scenario presents an unappetizing prospect for the long-term manufacture of the drug substance in question.

Alternatively, a company that invests to a greater degree in product and process understanding will potentially map a more differentiated territory: some potential quality attributes may be less critical to product performance than others, some parameters in the process description will be found to have a greater impact on product (step) quality than others, and some potential control points will be found to be either unnecessary to the overall control strategy or redundant to other controls. This will allow the exploration of parameter limits beyond the limits simply gained through manufacturing experience and allow the development of focused controls rather than those simply defined by batch history. The applicant is then better able to evaluate the true impact on product safety, quality, and efficacy across the life cycle of the product, being able to predict the effect of change rather than reactively testing the impact.

As well as the minimum requirements outlined earlier, ICH Q11 [4] also defines additional elements that should form part of an enhanced approach, these being

- A systematic approach to evaluating, understanding, and refining the manufacturing process including
  - Identifying, through prior knowledge, experimentation, and risk assessment, the material attributes and process parameters that can have an effect on drug substance CQAs
  - Defining the functional relationships that link material attributes and process parameters to CQAs of drug substance
- Using the enhanced approach in combination with quality risk management to establish an appropriate *control strategy* (which can, e.g., include a proposal for a design space)

Irrespective of whether an applicant establishes, and shares, an enhanced understanding of the process to manufacture the drug substance, the goal for all applicants is to establish a commercial manufacturing process capable of consistently producing drug substance of the intended quality. The process development program should identify which material attributes (e.g., of raw materials, starting materials, reagents, solvents, process aids, intermediates) and process parameters should be controlled. This may lead an applicant to assess the impact of variability in material quality attributes upon the quality of the drug substance. Certainly, appropriate specifications will be established to control each material as required to maintain its quality and support the maintenance of quality of the drug substance.

This evaluation of material attribute quality should also consider the impact of scale. Ultimately an applicant must ensure the understanding developed is pertinent to the proposed manufacturing scale and to any variability in the manufacturing process that is allowed in the process description.

23.4 Communicating the Drug Substance Development "Story"

The ICH Q11 [4] guidance notes that certain fundamental parts of the development of the drug substance are expected to be communicated in the CTD and gives advice on the expected content of section S.2.6 "Manufacturing Process Development." The applicant is asked to describe and explain significant changes that have been made during the development of the drug substance, and its manufacturing process, in particular, changes from the manufacture of pivotal toxicological, clinical, and stability lots to the proposed commercial manufacturing process to be run at full scale. This information is important to support the assessment of the drug substance as it highlights key changes made in the finalization of the commercial process and enables the assessor to understand any risks that need to be managed by the applicant. These risks can include potential scale dependence of the applicant's development understanding and any risks to the stability of the drug substance that may have not been suitably addressed by the registration stability program. The assessor will also be able to evaluate the changes in manufacturing process that have occurred during development and their impact, if any, on quality and contextualize these against the process optimization studies presented.

The applicant can also usefully use this section of the CTD to rationalize the manufacturing process description and associated controls that will become the "commitment" elements of manufacturing compliance for the future production of the drug substance. Thus the applicant can describe their approach to the manufacture of the drug substance, their approach to selection of starting materials (and the manufacturing suppliers and controls of these starting materials), and the approach taken to process development that has led to the understanding of the critical aspects of materials and process that assure the quality of the drug substance.

At one extreme, the development story might describe how a drug substance process was identified very early, prior to the manufacture of pivotal toxicology and clinical lots and used, without significant change, to support development studies and then scaled-up to support commercial supply, with tight controls on process parameters and input materials (these controls based on material specifications and process parameters used in development). This may sound unrealistic, but is not too dissimilar from the typical approach taken for the "traditional" development of some biological drug substances. In such circumstances, the quality of the product is considered to be intimately bound to the process and how it is performed, and little flexibility in manufacture, control, or quality is expected.

In contrast, development could also involve deliberately-engineered process change, potentially evaluated after crucial stages in development, for example, after key toxicology studies have been conducted that qualified the impurity

profile of the early development drug substance. In such circumstances, it would be important to reflect on the potential changes in quality of the drug substance that may result from the process change and to provide evidence that the resulting drug substance remains of an appropriate quality for use, perhaps by inclusion of further information on the specific qualification of impurities in the revised quality profile. In these cases, the applicant may have to rationalize the ongoing safety and quality of the drug substance and also justify the change made to the manufacturing process to explain why the change needed to be made to secure the supply of drug substance at scale in a viable manner.

Similarly, a change to drug substance manufacture might be made after establishing the registration stability studies. This change can call into question whether the drug substance that is being evaluated in these studies remains representative of the ongoing quality of the drug substance, an important factor for the review of the application. Thus, such changes can become matters for considerable scrutiny by the assessor, and the applicant should present such changes in a well-rationalized way in the application (section S.2.6).

Based on the anticipated regulatory scrutiny of changes, it is certainly advantageous that an applicant presents such changes in a way that shows how quality risk management principles have been applied to support the change.

Quality risk management may also be used in a more fundamental sense in the development of the manufacturing process and its associated control strategy. Such an approach will result in a more detailed and granular understanding of what factors specifically impact the quality of the drug substance. This understanding can lead to modifications in the details of the process and controls presented by the applicant in the submission to support ongoing manufacture.

For example, an applicant may develop a considerable understanding of how a CQA of the drug substance is established by the process and the role that the quality of input materials and process parameters within specific processing steps play in maintaining quality. This might lead to a proposal to control this CQA, not by end-product testing but by a combination of controls on input materials and process parameters. Such an approach may be taken for one or a number of CQAs associated with the drug substance and essentially provides a design space for the assurance of quality of each CQA.

It is also possible that such enhanced development approach can lead to different specifications being proposed for some ingoing materials than would be the case in the baseline approach outlined. An enhanced approach will BOTH likely focus controls on ingoing materials on critical aspects of their quality (not all possible aspects that might be specified in a baseline approach) AND perhaps provide for wider acceptance criteria than have been evaluated during development manufacture, based on a more comprehensive understanding of the linkage between the quality attribute of the ingoing material and the quality of the drug substance. This can involve understanding, for example, the fate and purge

of an impurity in an ingoing material and how this processing leads to the removal of the impurity. Such removal can be more efficient than exemplified in the limited amount of manufacturing experience accrued at the stage of application and thus can support wider specification acceptance criteria being proposed.

A further important consequence of enhanced approaches to development can be the identification of particular process parameters in manufacturing steps as being critically important to achieving the quality of the product from that step (and hence assuring the quality of the drug substance). In a baseline approach, the impact of any one parameter may not be well understood and hence all parameters need to be considered as potentially as critical as one another. And if no variability in process operation has been explored in a baseline approach, then parameters will need to be tightly bound to the ranges used in batch manufacturing experience. However, an applicant can use prior knowledge and focused experimentation to understand the differential impacts of various parameters associated with a process step, such that some will be found to need control to a greater degree than others. In addition, the variability around some critical parameters can be explored in a designed way to understand what actual operating flexibility may be possible for their operation during manufacture. Provided such understanding remains appropriate at the proposed manufacturing scale, these types of knowledge can impact both the number of parameters detailed in the manufacturing process description (excluding those parameters that are not critical to the quality of the step) and the degree of detail and operating ranges provided for each parameter. When this experimentation has been developed in such a way as to understand the interactions of various parameters (e.g., using multivariant experimental design), one can build a comprehensive understanding of the process step (a design space) and the controls and commitments (parameter ranges) needed to adequately describe the process.

One can appreciate that such enhanced approaches to development can have significant benefits for the applicant in terms of process understanding and in the design of optimal processes and controls. It is therefore critical for the applicant to describe their approach to development so that the assessor can fully appreciate what has been understood and also the scientific basis for the conclusions reached. Thus decisions made during development need to be transparent and, most importantly, understandable to the assessor for the assessor to be able to reach the same conclusions as the applicant on the appropriateness of the process description and controls for the manufacture of the drug substance.

23.4.1 Potential Challenges to an Enhanced Submission

As highlighted earlier an applicant may choose to employ risk assessment techniques to prioritize process inputs and/or to employ multivariant experimental design. These approaches are considered appropriate, but

every decision made by an applicant that leads to prioritization and de-prioritization of inputs for further investigation can lead to questions from the assessor about the basis of such decisions. Assessors are as interested in understanding how an applicant has assessed the criticality of process inputs as they are in understanding why an input has been considered noncritical. However a black-and-white critical/noncritical division is awkward. For example, an assessor may think that de-prioritized process inputs are not noncritical, only less critical. Such concerns would be further exacerbated if the classification of an attribute as noncritical leads an applicant to provide less information in the submission of manufacturing commitments related to noncritical attributes.

In addition an assessor can also question whether the experimental understanding and decisions made from experiments conducted at small scale remain relevant to full manufacturing scale.

It may look from an assessors perspective that "enhanced" development may be about applicants making decisions to reduce "commitments," that every decision carries some degree of residual risk or uncertainty, and that operational flexibility/design space established on the basis of "enhanced" development experiments conducted at less than full manufacturing scale may be flawed or carry undefined levels of risk to quality. Thus, one can appreciate that there could be regulatory concern about simply empowering applicants to have the freedom to change operating conditions based on "enhanced" development "understanding" within a designated design space. Certainly, assessors faced with a plethora of different approaches to development and different proposals for process and control commitments based on these development approaches are in a challenging situation, given the role of the regulator is to ensure that a product will be appropriately manufactured and controlled.

How might an assessor cope with this challenge? One option might be for an assessor to become very diligent (even conservative) in their assessment of "enhanced" proposals for manufacture and control, where every decision and approach is queried and every piece of data and its conclusion is risk assessed. One often hears that an applicant should make an assessor aware of the rationale for every experiment and the data that supports every conclusion and decision. This might lead an assessor feeling the need to be responsible for every detail and thus to question, challenge, or tighten every proposal/element of a submission.

Such a situation might be avoided were the assessor ultimately confident that the control strategy applied to a drug substance and its manufacturing process was fully appropriate to assure quality. Thus, the control strategy that derives from the product and process understanding presented may be pivotal in assessing an application.

23.5 Control Strategy Development

There is a need for careful linkage of control strategy elements and process understanding in ICH Q11 [4], perhaps to a greater degree than in Q8 and for drug product, as the CQAs of drug substances are not the only important aspects of control attended to by the manufacturer of the drug substance. Control points for important attributes of drug substance quality can also be at intermediates or starting materials stages rather than exclusively at the final drug substance stage. In this way, a drug substance manufacturer can truly build quality in, by controlling important aspects of starting material, reagent, and/or intermediate quality such that risks to drug substance quality (e.g., potential impurities in drug substances) can be controlled without recourse to/reliance on end-product testing. Thus Q11's focus on drug substance CQAs as being properties that should be within an appropriate limit, range, or distribution to ensure the desired product quality is not incorrect but, if taken too literally, provides a rather blunt perspective on how the quality of the drug substance can be assured. It is important to remember that ICH Q11 [4] (section 3.1.5) notes that "manufacturing process development should identify which material attributes and process parameters should be controlled" in the control strategy. This better reflects the value and importance of the control strategy in drug substance development.

It is also important to reflect that, for drug substance manufacture, some potential risks to quality, such as impurities present in starting materials, can be significant in the overall understanding of quality but that a considered position on such a risk can be reached by considering both controls on such an impurity at the starting material (or an intermediate) and the effect of subsequent processing on the impurity in question. The applicant can understand whether the impurity would react in further processing and thus should be evaluated as a derived "fate" product (perhaps with different associated risks, i.e., a mutagenic impurity can be reactive and be converted to an impurity of lesser reactivity and toxicity by the standard process). The applicant can also understand whether an impurity is purged (removed) during standard processing, either in a reactive manner (e.g., a basic impurity removed by a designed acid wash) or as a consequence of its physical properties (which might allow for an impurities removal into, e.g., mother liquors of an isolated solid intermediate, on the basis of solubility). All of these factors, based on inherent chemical properties of impurities and desired materials and understanding of chemical reactivity, allow a process chemist to be innovative and address potential risks to drug substance quality in ways not available to drug product manufacturing scientists. This means that the development of appropriate controls for drug substance manufacture can have more options for the optimal placement of controls based on product and process understanding than for drug product attributes.

ICH Q11 states that "risk assessment can be used during development to identify those parts of the manufacturing process likely to impact potential CQAs" and that, in an enhanced development approach, the determination of appropriate material specifications and process parameter ranges could follow a sequence such as:

- Identification of potential sources of process variability.
- Identification of material attributes and process parameters likely to have the greatest impact on drug substance quality.

  This does not clarify what should be done with those material attributes or parameters not likely to have a significant impact on drug substance quality—should these be subject to less regulatory oversight? Should they be described in the manufacturing process description at all? If described, how can those of lesser importance be clearly differentiated?
- Designing and conducting of studies (mechanistic/kinetic evaluations, experiments, simulations, modeling) to identify links between material attributes, process parameters and drug substance CQAs.

  This helpfully, and appropriately, suggests that approaches other than experimentation can sometimes be used to develop the process and process understanding. This however either does not clarify how the assessor can be assured of the veracity of such approaches nor does it clarify how "exact" such understanding needs to be if and when the resulting risk to quality is appropriately managed in the overall control strategy. Of course, it is important for the manufacturer to understand a process well in order to operate it robustly without having batches of drug substance fail controls but for the assessor to have to both ensure the suitability of controls AND fully interrogate and underwrite the manufacturer's process understanding may be too onerous a task. Moreover, this might be a complication that adds no real assurance of the quality of the product if the product's quality is suitably assured by an appropriate, agreed control strategy.
- Analysis and assessment of the data to establish appropriate ranges, including establishment of a design space if desired.

In the simplest case, drug substance made by the process as operated in development will meet specification (at least at that manufacturing scale). As any widening of any operating range beyond the company's experience is proposed, the manufacturer needs to ensure that the more flexible process will continue to produce drug substance that meets specification. On one level, the regulation of the process continues by maintenance of the specification/process controls. However, the regulatory responsibility extends across the life cycle of the product and thus is required to manage change. As an applicant widens the operating conditions of a process beyond precedent and creates more opportunity for self-managed change (e.g., freedom of change within an

agreed design space), then the regulatory responsibility may be viewed to extend to assurance of the scientific foundation of the operating space to maintain appropriate oversight of change as required in the pharmaceutical regulations.

The more operational flexibility that an applicant proposes in a submission, the more the assessor is under obligation to understand not only the control strategy but also the scientific basis for the operational flexibility claimed. As post-approval changes often require provision of stability data (or even PK (pharmacokinetic) study/comparability data), one can see that an assessor may seek assurance not only of drug substance release quality (including appropriateness of analytical methodology to monitor change) but also assurance of stability and biopharmaceutical/*in vivo* performance. Thus, an applicant may expect to have to provide data or strong justification for release quality, stability performance, and *in vivo* performance in support of a design space. This can make a "design space" difficult to establish, especially for complex products/substances (where there are multiple CQAs and the linkage to process inputs may not be fully understood) or unstable materials.

An applicant can expect to have to balance this challenge by investing not only in a robust control strategy that assures quality at release even through potential change but also in an effective understanding of product stability (i.e., what factors assure appropriate stability or lead to significant instability) and the product's *in vivo* performance.

For small molecules, *in vivo* performance can be characterized via attributes on the drug substance/drug product release specifications to some degree, but for biological molecules some facets of *in vivo* performance/comparability are more difficult to link to attributes that can be controlled at release. Thus for a biological drug substance, there can remain more difficulty in building processing flexibility into manufacture and more reliance in or trust of "the process assures the quality of the drug substance." ICH Q11 [4] states that "the development of a Design Space for some biotechnology/biological drug substances can be challenging…" and suggests that, depending on the level of residual risk, it may be appropriate for an applicant to provide proposals on how movement WITHIN a design space would manage post-approval, even though "working within the Design Space is not considered as a change."

Requests from assessors to understand how changes will be managed within a design space have also been received during assessment for noncomplex, small molecule submissions. ICH Q11 suggests that the change management proposals "should indicate how process knowledge, control strategy and characterization methods can be deployed to assess product quality." Thus, the balancing factor to risk within a design space is, again, the control strategy, perhaps with augmented "second tier" risk assessment and testing.

23.6 The Established Control Strategy

The guideline has placed considerable emphasis on the concept of the control strategy as a pivotal aspect of manufacturing commitment and assurance of quality. It is therefore germane to examine how ICH Q11 [4] describes "control strategy" as a "planned set of controls, derived from current product and process understanding that assures process performance and product quality." Every drug substance manufacturing process, whether developed through a traditional or enhanced approach, will have an associated control strategy.

A key aspect of this definition and expectation is that the process, as well as the drug substance manufactured, should be "under control." Thus, a control strategy is more than the drug substance specification.

Under Q11, a control strategy is stated to be inclusive of (but not limited to)

- Controls on drug substance
- Process parameter controls and other controls implicit in the design (and description) of the manufacturing process
- Controls on other materials associated with the process (e.g., raw materials, reagents, starting materials, etc.) and the drug substance (e.g., primary packaging materials)

The more an applicant invests in (and shares) process and product understanding to determine what factors need to be controlled to limit variability in product quality, the more focused can be the applicant's proposed controls. Such focused, enhanced controls are likely to place less emphasis on drug substance testing and more emphasis on parameter and input material controls. One might imagine the extreme case of a drug substance manufactured within a design space where every CQA is assured parametrically rather than by testing and thus the drug substance is released on the basis of compliance with manufacturing commitments (i.e., made within the design space) alone. However, a more likely eventuality for many drug substances is that CQAs will continue to be assured through testing of the CQA (or an upstream progenitor of the drug substance CQA) and not purely on the basis of process compliance. Therefore, such associated controls can be seen to balance risk of manufacturing uncertainty and non-compliance. Such a balance should allow an assessor to risk assess a submission to understand how a process description and control strategy work together to assure quality and perhaps focus review attention specifically on seeking to evaluate where the control strategy and process description together do not provide adequate assurance of ongoing quality and are therefore deficient and require revision/enhancement.

Clearly it is more straightforward, especially for the assessor, if the desired drug substance CQA is controlled on the drug substance specification with an acceptance criterion directly associated to safety and/or efficacy and rationalized

accordingly. However it is also legitimate and appropriate to control a CQA by testing the attribute at an earlier intermediate stage to the same acceptance criterion. In this case, the assessor has to be able to conclude that the attribute is suitably controlled and will not be subject to further adverse change as the manufacture of the drug substance proceeds. So, even in this very simple "enhanced" case, one can start to see the strain begin to be placed on "process understanding" and on the assessor, who is charged with ensuring the control strategy fully and appropriately controls the quality of the drug substance. As "enhancements" to the control strategy continue, these strains continue to grow.

It is also possible for legitimate controls to do the following:

1) Control a CQA at an earlier intermediate but at a higher acceptance criterion, based on the understanding of purge effected in downstream processing. Such an option is defined within ICH M7 [11] option 3, relating to control of mutagenic impurities.
   Assessor concern/process understanding—Is the understanding of purge at different scales and under different operating conditions robust? Is the control based on an understanding of the "worst case"?
2) Control a progenitor of the CQA at an earlier intermediate based on an understanding of "fate" of the progenitor attribute.
   Assessor concern/process understanding—Is the "fate" understanding robust? Should the control strategy include further testing downstream for either material?
   These can be control strategies of particular value if the "progenitor" is, for example, a mutagenic material that is known to be transformed to a non-mutagenic material in downstream processes.
3) The applicant may even propose to drop testing for a CQA, either because of "purge" knowledge or on the basis of parameter control.
   Assessor concern/process understanding—How will this strategy maintain quality against process change?

One can appreciate that an enhanced control strategy can easily draw on commitments and understanding presented in different places across the submission, as formatted in CTD structure. This can lead to significant complications for applicant and assessor alike in presenting and ascertaining the comprehensiveness of the control strategy presented. An applicant may therefore consider it very valuable to provide a summary of the "control strategy" for the manufacture of a drug substance summarizing the various aspects of control for each CQA. This further demonstrates the necessity for applicants to tell a coherent control strategy story that facilitates assessor review.

It is also clear that control strategy development quickly becomes linked to change management and the need to operate a "validated" process that has been demonstrated to be routinely capable of manufacturing drug substance of the desired and appropriate quality.

23.7 Process Validation

Whereas process validation is barely addressed in ICH Q8 [1], this is a topic where guidance is specifically provided in ICH Q11 [4] and again the guidance is complex, being differentiated by both drug substance and by degree of product understanding. For biological/biotechnological products and sterile drug substances, an applicant needs to provide specific validation information in the manufacturing application. This is not required in the case of non-sterile small molecule drug substance; however in all cases, the manufacturing process for the drug substance should be validated before commercial distribution of the resultant drug products. The approach taken to this validation of the process can vary dependent upon the complexity, scale dependence, variability, and process understanding of the manufacturing process.

Validation is defined based on the principles articulated in ICH Q7 [12]. It is required that the applicant provides documented evidence of the capability of the process to "effectively and reproducibly" produce the drug substance meeting predefined quality requirements, for example, predetermined specifications based on identified CQAs (the "what"), but the manufacturer has considerable flexibility, in principle, in *how* this assurance is provided.

In many cases, applicants almost default to a "traditional" approach to process validation, wherein a series of consecutively manufactured full-scale batches are used to demonstrate, through intermediate and end-product testing, the quality (and variability in quality) of the drug substance produced by a fixed process. Often, three batches are made to fulfill this purpose. However, this "traditional" approach can be substituted by approaches where validation may be conducted at reduced scale (if an operation is known to perform in a reproducible and predictable manner irrespective of scale) or by employing a continuous verification approach where, essentially, the process knowledge and control strategy are so well understood that each lot of the drug substance can be predicted to provide appropriate quality. Such a knowledge-intensive approach can be very useful when operating continuous manufacturing processes and can also support change management, including "flexible/adaptive" processing wherein process parameters can be adjusted to account for significant variabilities in the input quality of materials.

There is considerable focus on assuring that changes in process and scale of manufacturing are managed to continue to assure quality, but there is also recognition in ICH Q11 [4] that certain studies conducted at small or pilot scale can contribute to the overall validation of the normal process (or of process variability). Such studies can allow for modeling of the manufacturing process at full scale and can reduce the dependence on a number of full-scale lots needed to validate the process (or process variants), provided the understanding generated at small scale can be demonstrated to be linked to the operation of

the process at full scale. This can support, at one extreme, a process being shown to be valid without any data from running the process at full scale, based on the process understanding and controls in place.

It should also be noted that the validation of a drug substance process, perhaps unlike most drug product processes, also needs to consider the removal of known and potential impurities. For biotechnological drug substances, the removal of viral contamination is usually a platform-type issue, with common challenges, and, here, common solutions can be extrapolated between different drug substances, and viral removal can be demonstrated at less than full manufacturing scale. However, in the manufacture of small molecule drug substances, impurities are more specific to the individual process and drug substance. For this reason, control strategies applied to such impurities have to be carefully established on a case-by-case basis. The knowledge of the ability of a process to purge impurities, which may have been studied on small or pilot scale, may need to be reevaluated as scale of manufacture increases. This would be particularly important where tests for potential impurities are dropped from a control strategy based on small scale results. The most "at-risk" situation to consider is if an analytical test used at small scale has a particular ability, that is, specificity, to test for the presence of certain impurities, but the analytical test is modified on the basis of small scale data to remove the method's ability to resolve and thereby test for those impurities. Such evaluations can be augmented by the of-risk assessment tools designed to evaluate the purging of impurities [13].

The validation of a manufacturing process is a GMP requirement and is conducted from the entry point of the agreed starting material to the synthesis. Thus, the selection of the starting materials for drug substance manufacture becomes a pivotal element of the regulation of a drug substance manufacturing process.

23.8 Selection and Justification of Starting Material for Drug Substance Commercial Manufacturing

The selection and agreement of the starting materials and the assurance of their quality for manufacture is another complex topic included in ICH Q11 [4].

The EU had some existing guidance [14] on drug substance starting material expectations and some thinking had been published from the FDA in the (now withdrawn) FDA Draft Guidance on Drug Substance [9]. Nevertheless, this was a topic where guidance was needed as regulatory expectations were not harmonized. Stakeholders also recognized that case-by-case considerations arose in the selection of starting materials for drug substance manufacture and in their evaluation by regulators.

Again the ICH Q11 [4] guidance had to cover both small-molecule drug substances and biotechnology-derived drug substances, as well as drug substances derived from natural materials by semisynthesis. Each differs with respect to how starting materials should be considered.

The EWG most likely have found this an interesting set of topics to consider, and there would likely have been some discussion of various underlying principles of the selection expectations and how these could be provided in the guidance. For example, regulators had historically accepted proposed starting materials that are commodity materials (i.e., those that are available commercially with significant non-pharmaceutical use). The eventual Q11 guidance would also probably want to reflect this acceptability BUT without wanting to stipulate that all proposed starting materials had to be commodity materials (not the case historically) and without suggesting that any commercially available material might be acceptable (as some proposed starting materials can be provided to the API manufacturer by a contract manufacturer under license, and this would not meet the regulator's perspective on preexisting material supply). Thus the guidance had to find ways to allow for consideration of all these factors.

Furthermore, there have often been questions about "how close/far does a proposed starting material have to be from the drug substance to be a suitable and acceptable starting material?" While this is a question that seems appealing to provide a simple answer to, a simplistic approach is very likely to fall short of expectations for a variety of reasons, leading to a rigid and ineffective regulatory framework. So how could Q11 provide guidance on this topic, suitable to cover the breadth of product types and address the range of concerns described earlier?

The ICH Q11 [4] guidance contains, in section 5, a number of subsections, providing guiding principles for the selection, justification, and submission of documentation relating to starting materials (and source materials) for drug substance commercial manufacturing:

- General Principles for the Selection of Starting Materials for Synthetic Drug Substances
- General Principles for the Selection of Starting Materials for Semi-synthetic Drug Substances
- General Principles for the Selection of the Source and Starting materials for Biotechnological/Biological Drug Substances
- Submission of Information for Justification of Starting Material Selection for Synthetic Drug Substances
- Submission of Information of Starting Material Selection for Semi-Synthetic Drug Substances

In addition, the reader is referred to the ICH guidances Q5A [15], Q5B [16], and Q5D [17] for the qualification of source of starting materials for biotechnological/biological drug substances.

For the selection of starting materials for synthetic drug substances, the Q11 text avoids stating any "absolute" rules for selection and instead provides a number of general principles that should be considered by the applicant in positioning their selection decision. No one of these principles stands as an absolute requirement, and it is expected that an applicant should consider all of the principles to arrive at a balanced proposal.

The principles are science- and risk-based focused on providing quality assurance of the derived drug substance and allowing for the review of potential risks to drug substance quality by the regulators.

These principles are reproduced here for convenience:

- In general, changes in material attributes or operating conditions that occur near the beginning of a manufacturing process have lower potential to impact the quality of the drug substance.
- The relationship between risk and number of steps from the end of the manufacturing process is the result of two factors, one concerning the physical properties of the drug substance and the other concerning the formation, fate, and purge of impurities. The physical properties of a drug substance are determined during the final crystallization step and subsequent operations (e.g., milling, micronization), all of which occur at the end of the manufacturing process. Impurities introduced or created early in the manufacturing process typically have more opportunities to be removed in purification operations (e.g., washing, crystallization of isolated intermediates) than impurities generated late in the manufacturing process and are therefore less likely to be carried into the drug substance. However, in some cases (e.g., when peptides or oligonucleotides are synthesized on solid support), there is a more limited relationship between risk and number of steps from the end of the manufacturing process. Indeed in the case of oligonucleotides, an impurity present in a mononucleotide starting material can be amplified in the final API if, this is as often is the case, it utilized at several stages in the construct of the oligomer.
- Regulatory authorities assess whether the controls on the drug substance and the drug substance manufacturing process can be considered adequate, including whether there are appropriate controls for impurities. To conduct this assessment, *enough* of the drug substance manufacturing process should be described in the application for regulatory authorities to understand how impurities are formed in the process, how changes in the process could affect the formation, fate, and purge of impurities, and why the proposed control strategy is suitable for the drug substance manufacturing process. This will typically include a description of multiple chemical transformation steps.
- Manufacturing steps that impact the impurity profile of the drug substance should normally be included in the manufacturing process described in section S.2.2 of the application. (But see example 4 in ICH Q11 [4] that shows how the control strategy applied can balance such expectations.)

- Each branch of a convergent drug substance manufacturing process begins with one or more starting materials. The GMP provisions described in ICH Q7 [11] apply to each branch beginning with the first use of a starting material. Performing manufacturing steps under GMP together with an appropriate control strategy provides assurance of quality of the drug substance.
- A starting material should be a substance of defined chemical properties and structure. Non-isolated intermediates are usually not considered as appropriate starting materials.
- A starting material is incorporated as a significant structural fragment into the structure of the drug substance. "Significant structural fragment" in this context is intended to distinguish between starting materials from reagents, solvents, or other raw materials.

Given the number of aspects that deserved to be considered in putting together guidance on this topic, these principles seemed to be very appropriate, with their focus on understanding criticality, impurity formation and purge, and on provision of information to the regulators. One can readily see the careful consideration of scientific aspects that should be considered in reaching a selection decision, and one can see how factors that affect risk to drug substance quality are also built in. Nevertheless, one can probably also predict that any two applicants could make very different proposals (either or both of whom might be accepted or rejected based on the justification provided by the applicant). Regulators would be left to manage a rather divergent outcome and have to ask questions of applicants about how their proposal meets/considers all the expectations built into the principles. This might often seem to an applicant to result in requests to lengthen regulated processes to a particular or longer number of steps. Thus the principles in ICH Q11 [4] while science based and built on risk-management principles might also lead to significant implementation challenges for regulators and industry alike.

For example, an applicant who has made a proposal for a starting material in their marketing application might find it challenged or turned down in at least one region during review. There might not be consistent regulatory positions on whether the proposal is acceptable and maybe different remediation actions requested of the applicant by the regulators. Thus one could find a proposal intended to facilitate global alignment ultimately resulting in specific regional requirements. Furthermore, if the applicant has proceeded to manufacture launch stocks for initial commercial supply from the proposed starting materials, the applicant could find that these stocks are not considered suitable in any region that rejects the proposed starting materials. It would be unfortunate if this alone was to prevent a new medicine reaching the patients, but there may be no way for a regulator to allow the initial supply to be used and still request remediation of concerns with the proposed starting material. To manage this risk of regulatory rejection, applicants may employ contingency positions (of validating longer syntheses for initial supplies and/or conducting additional

steps under GMP), and while these are not unreasonable risk management positions, they introduce complexity into planning for launch stocks. Such contingent actions could be avoided if the acceptability of a starting material proposal either was more predictable or could be agreed with regulatory agencies globally in a simple manner prior to marketing application submission and prior to committing to the manufacturing decisions for launch. (Such challenges became the focus for the ICH Q11 IWG established in 2014.)

Certainly, the applicant will want to ensure that the proposal for the starting materials is communicated in the marketing application in a way that regulators can appreciate and properly evaluate. Section 5.2.1 of ICH Q11 [4] notes that the applicant should "provide a justification for how each proposed SM is appropriate in the light of the general principles," and this should encourage the applicant to be transparent with their reasons and rationales for the selection proposal made and with an analysis of how their control strategy proposed (e.g., for the starting material specification) helps assure drug substance quality.

ICH Q11 provides guidance that such justification could include information on the capabilities of analytical methods used to detect impurities in the starting materials, the applicant's understanding on the fate of impurities, and what specification controls are needed to assure the quality of the drug substance.

Sometimes an applicant may be faced with questions about how their proposed starting material or controls manage change in the manufacture of a starting material (remember this manufacture is not regulated) and may wish to give consideration to how to manage such concerns when working with their starting material suppliers.

Alternatively, the applicant may find that the regulator is concerned about whether there could be cross-contamination risks present in the starting material that could impact drug substance quality. Again, the applicant may wish to give consideration to how to address such concerns when working with their starting material suppliers.

The ICH Q11 [4] guidance on the manufacture of semisynthetic drug substances is also helpful. Traditionally, the start of such a manufacturing process has been considered to be the natural source of the chemical materials, for example, the plant rather than the isolate and the cell bank rather than the isolated fermentation product. ICH Q11 has modified this expectation to recognize that the isolated chemical material (rather than the natural source) can be an acceptable starting material to propose for a semisynthetic drug substance if the isolated chemical can be shown to meet the general science-/risk-based selection criteria. If such a case cannot be made to the satisfaction of the regulator, the applicant would be asked to describe the manufacturing process starting from the natural source material.

For biotechnological/biological drug substance, Q11 continues to expect that the input cell banks are the starting points for the manufacture of biotechnological drug substances and some biological drug substances. Science and understanding in this field is changing, and there may be cases to be made in future for different approaches. For example, antibody drug conjugates are drug substances where an antibody is chemically linked to a second material with pharmacological activity. The linker is likely made by chemical synthesis and the conjugated material may be either synthetic or semisynthetic. In such an instance one may have starting materials that are cell lines (for the antibody) and starting materials that are either natural sources or isolated chemicals (for the semisynthetic conjugate) as well as the chemical linker that may be considered as a starting material in its own right (if so justified) or may need earlier steps in its synthesis described to satisfy the regulators perspective on risk to drug substance quality.

This guidance on starting materials is a pivotal part of the Q11 content and constitutes a significant part of the work of the EWG. Seeing the justification of SM as part of the critical aspects of the quality story and control strategy is important to regulators and applicants alike.

23.9 Summary/Conclusions

ICH Q11 [4] provides harmonized expectations for the development and registration of drug substance manufacturing processes and control strategies and in particular shows how these development expectations are linked to achieving aspects of drug product quality, safety, and efficacy. To be able to provide such guidance in such a way as to cover both small molecule and biotechnological drug substances, the guidance often operates at an "in-principle" levels, conceptualized above the scientific details of chemistry and biotechnology. This brings the potential issue that the registered information and the assessor is left struggling. The guidance recognizes that considerable "enhanced" scientific understanding can underpin an applicant's submission and suggests some potential value of taking the option to provide an enhanced level of information, in terms of optimized registered details and life cycle change management opportunities. However, there may be considerable challenges in being able to deliver a submission that clearly and comprehensively captures the depth of an applicant's understanding to gain this enhanced regulatory standing, and there may be considerable questions related to risk assessment (and risk acceptance or risk management) conducted by the applicant in developing the scientific understanding.

It may be possible for an applicant and an assessor to have different expectations of the balance between scientific understanding and controls put in place

to assure the quality of the drug substance, such that questions related to science or experimental design result even when an attribute is suitably and fully assured by "end-product" controls and testing, as regulators seek to "build-in" quality. Given this, there may be a diminishment in return for both applicant and regulator in sharing the underlying science behind a submission, beyond establishing a strong and mutual understanding of the basis of controls that assure quality, and support to life cycle management in effective ways.

Nevertheless, an applicant who has chosen to take an enhanced approach to development (i.e., having understood the various factors that can impact upon delivering the required quality of drug substance) will anyway gain considerable benefits for their manufacture of a drug substance, as manufacturing will likely be more robust, more efficient, and more plannable, with a better understanding of the required quality from suppliers of important ingoing materials and an increased predictability in terms of supply and medicines availability. In this way, scientific and risk-/understanding-based approaches to manufacture can bring significant benefits to all stakeholders reliant upon effective manufacture of pharmaceuticals. It is not a matter of "what can a manufacturer gain from a regulator" from the science presented, but what the scientific understanding enables a manufacturer to deliver in terms of improved robustness and process capability. In this way, the manufacturer can convert having made the appropriate regulatory commitments from a "regulatory risk" of non-compliance and product failure into a business advantage.

References

1 ICH Q8. Pharmaceutical Development http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q8_R1/Step4/Q8_R2_Guideline.pdf. Accessed on April 10, 2017.
2 ICH Q9. Quality Risk Management http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q8_R1/Step4/Q8_R2_Guideline.pdf. Accessed on April 10, 2017.
3 ICH Q10. Pharmaceutical Quality System http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q10/Step4/Q10_Guideline.pdf. Accessed on April 10, 2017.
4 ICH Q11. Development and Manufacture of Drug Substance http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q11/Q11_Step_4.pdf. Accessed on April 10, 2017.
5 ICH M4Q(R1). The Common Technical Document for the Registration of Pharmaceuticals for Human Use: Quality—M4Q(R1) Quality Overall Summary of Module 2 Module 3: Quality http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/CTD/M4_R1_Quality/M4Q__R1_.pdf. Accessed on April 10, 2017.

6 ICH Q6A. Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q6A/Step4/Q6Astep4.pdf. Accessed on April 10, 2017.

7 ICH Q6B. Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q6B/Step4/Q6B_Guideline.pdf. Accessed on April 10, 2017.

8 EMA. Note for Guideline on Development Pharmaceutics http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003533.pdf. Accessed on April 10, 2017.

9 Guidance for Industry. Drug Substance Chemistry, Manufacturing, and Controls Information http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/ucm052498.pdf. Accessed on April 10, 2017.

10 Guidance for Industry. Drug Product Chemistry, Manufacturing, and Controls Information http://www.fda.gov/OHRMS/DOCKETS/98fr/02d-0525gdl00001.PDF. Accessed on April 10, 2017.

11 ICH M7. Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M7/M7_Step_4.pdf. Accessed on April 10, 2017.

12 ICH Q7. Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q7/Step4/Q7_Guideline.pdf. Accessed on April 10, 2017.

13 Teasdale A, Elder D, Chang S-J, et al. Risk assessment of genotoxic impurities in new chemical entities: strategies to demonstrate control. *Org Proc. Res Dev* 2013, 17: 221–230.

14 EMA. Guideline on the Chemistry of New Active Substances CPMP/QWP/130/96. Accessed on April 10, 2017.

15 ICH Q5A(R1). Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q5A_R1/Step4/Q5A_R1__Guideline.pdf. Accessed on April 10, 2017.

16 ICH Q5B. Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of R-DNA Derived Protein Products http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q5B/Step4/Q5B_Guideline.pdf. Accessed on April 10, 2017.

17 ICH Q5D. Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q5D/Step4/Q5D_Guideline.pdf. Accessed on April 10, 2017.

24

ICH M7

Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk

Andrew Teasdale

AstraZeneca, Macclesfield, UK

24.1 Introduction

ICH M7 [1] is one of the most recent ICH guidelines published, reaching step 4 in June 2014. The guidance itself is a multidisciplinary guideline reflecting the fact that mutagenic impurities are both a quality and safety topic. It focuses on the control of mutagenic impurities and was introduced to address differences that existed between regional guidelines pertaining to genotoxic (mutagenic) impurities, the principal regional guidelines being the European Medicines Agency (EMA) guideline [2], supplemented by its associated Q&A document [3] and the draft Food and Drug Administration (FDA) guideline [4]. No formal guidance was ever published by PMDA, Japan. A comprehensive overview of the chronological development of the EMA and FDA draft guidelines is outside the scope of the chapter; however such an overview is provided by Teasdale and Ogilvy [5]. These guidelines were introduced to address a perceived gap in existing impurity guidelines ICH Q3A [6] and Q3B [7] relating to the management of DNA reactive mutagenic impurities that may arise during the synthesis of the active, or form through degradation of either the drug substance (DS) or drug product (DP). Although broadly similar within their concept, there were nevertheless sufficient enough differences between the EMA and FDA guidelines to render a global approach challenging.

Clear within the ICH M7 concept paper [8] was a desire to harmonize these guidelines and to specifically address areas of uncertainty. Critical areas identified within the concept paper included:

1) Use of *in silico* structure–activity relationship (SAR) tools for the assessment of mutagenic potential.

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2) What are acceptable levels of genotoxic impurities during drug development?
3) What are acceptable levels of genotoxic impurities for marketing?
4) Should those impurities be regulated differently that are likely to have threshold effects?
5) Should levels of genotoxic impurities be regulated using a threshold of toxicological concern (TTC) approach?
6) Structurally related genotoxic impurities are likely to have similar mechanisms of action. Should these be summed in calculating a TTC?
7) What process of qualification testing should be followed for impurities that are metabolites?
8) What additional data are needed to support having no special restrictions, or a higher acceptable daily intake (ADI) than the TTC, for a genotoxic impurity?

The following chapter will look in detail at the ICH M7 guideline, examining each aspect of the guideline, seeking in doing so to give a comprehensive overview of its practical implementation. It will also examine whether or not the guideline has succeeded in addressing the critical areas outlined in the concept paper.

24.2 ICH M7

The format and structure of ICH M7 is understandably very similar to that of the preceding regional guidelines, with specific sections focused on quality and safety aspects, although terminology does differ. Crucially the fundamental concept upon which the guideline is predicated has not changed; the principle of a TTC, that is, a virtually safe dose (VSD), remains the underpinning principle upon which the guideline is based. In addition to the overall framework there are additional sections intended to provide definitive guidance in specific areas, including:

1) Established products
2) Documentation
3) Case studies

These and other sections are now described in detail in the following text.

24.2.1 Introduction

The introduction, section 1 of the guideline, provides an overview of the relationship between the guideline and the closely related quality guidelines, ICH Q3A [6] and Q3B [7]. It also makes clear that the focus of the guideline is on DNA reactive mutagenic impurities, emphasizing for the first time the specific focus on such impurities as opposed to the more general group of

genotoxic impurities. This is a point returned to in more detail in the general principles section.

24.2.2 Scope

The scope (section 2) seeks to outline when and where the guideline is applicable, both in terms of previously established products (predating the introduction of the guideline), anticancer treatments, and therapy class.

24.2.2.1 Established Products

Previous guidelines, especially the EMA [2] guideline, have struggled to address the thorny question of the potential retrospective application of the guideline to existing products. This had led to variable interpretation, often resulting in requests for application to existing products triggered by apparent trivial changes (in the context of effect on levels of mutagenic impurities within the DS/DP) such as change in dose size and formulation volume and even in one case a change in address (name of the street changed) of the manufacturing facility! A lot of the uncertainty related to the inclusion within the original EMA guideline of a catch-all phrase of "cause for concern." Attempts to resolve these uncertainties made via the EMA Q&A process [3] proved only partially successful.

In contrast ICH M7 is clear and concise. Existing products are only in scope where:

1) Changes to the DS synthesis result in new impurities or increased acceptance criteria for existing impurities.
2) Changes in the formulation, composition, or manufacturing process result in new degradants or increased acceptance criteria for existing degradants.
3) Changes in indication or dosing regimen are made, which significantly affect the acceptable cancer risk level.

It is hoped that this addresses much, if not all, the uncertainty that has existed previously. One remaining area is how this applies to products marketed after the introduction of regional guidelines, particularly the EMA guideline, as this was formally issued and became effective in January 2007. Although not stated directly many have assumed such products, marketed after the introduction of earlier regional guidelines, to be in scope.

24.2.2.2 Anticancer Treatments

A challenging question has long been what is the relationship between guidelines pertaining to mutagenic impurities and the ICH S9 [9] guideline—Nonclinical Evaluation for Anticancer Pharmaceuticals? ICH S9 lays out clear principles relating to the nonclinical (i.e., toxicological) evaluation of impurities present within an anticancer agent. Specific within this is mutagenic impurities, S9 making clear that control to levels defined by EMA, FDA, and now

ICH M7 guidelines are not appropriate. ICH M7, within section 2 (scope), makes it clear that ICH S9 holds primacy.

The only remaining uncertainty relates to what is an acceptable level for a mutagenic impurity. This is not expressly defined. ICH S9 states, "Such limits are not appropriate for pharmaceuticals intended to treat patients with advanced cancer, and justifications described above should be considered to set higher limits." Many have interpreted this to mean that you can default to ICH Q3A/Q3B; however in reality it is likely that limits will be established on a case-by-case basis, with levels typically lower than ICH Q3A/3B but higher than defined in ICH M7 being agreed.

The scope section also addresses the scenario whereby the therapeutic agent in question is itself genotoxic. Importantly and, it is assumed deliberately, the wider term genotoxic is chosen, as opposed to the specific term mutagenic. Hence this encapsulates pharmacologically active agents whose mechanism of action can be described as genotoxic, for example, inhibition of cell proliferation, topoisomerase inhibition. The guideline specifically states that:

> Exposure to a mutagenic impurity in these cases would not significantly add to the cancer risk of the drug substance. Therefore, impurities could be controlled at acceptable levels for non-mutagenic impurities.

However, there exists an equally important question: "Does ICH M7 apply to other noncancer therapeutic disorders (e.g., rare diseases) where there is a significantly reduced life expectancy, that is, <2 years (i.e., life-threatening disorders, with a poor prognosis and with a similar risk benefit as is seen with cancer indications)?" The logical answer would be yes, but in the absence of an "ICH S9-like" guidance for rare diseases, this will require discussion with authorities on a case-by-case basis.

24.2.2.3 Nature of Therapeutic Agent/Excipients

The guideline makes clear that an assessment of the mutagenic potential is not required for the following types of DS and DP:

- Biological/biotechnological, peptide, oligonucleotide, radiopharmaceutical, fermentation products, herbal products, and crude products of animal or plant origin

Close scrutiny of this highlights some anomalies; certainly many peptides and oligonucleotides are synthetic/semisynthetic in nature, and it is perhaps difficult to rationalize their exclusion (indeed there was some commentary about these classes in the penultimate draft version of the guideline).

Established excipients are confirmed as also being out of scope. However, the guidance does indicate that it may be applicable for new synthetic excipients:

"The safety risk assessment principles of this guideline can be used, if warranted, for impurities in excipients that are used for the first time in a drug product and are chemically synthesized."

Similarly, the guidance clearly indicates that while not formally applicable to leachables the principles may be applied: "Application of this guideline to leachables associated with drug product packaging is not intended, but the safety risk assessment principles outlined in this guideline for limiting potential carcinogenic risk can be used, if warranted."

24.2.3 General Principles

Key within this section (section 3) is the reaffirmation of the specific focus of the guideline on mutagenic impurities. Indeed the guideline goes further in making the specific statement that:

> Other types of genotoxicants that are non-mutagenic typically have thresholded mechanisms and usually do not pose carcinogenic risk in humans at the level ordinarily present as impurities.

It also makes clear that in assessing mutagenic potential, this can be achieved through a combination of *in silico* SAR evaluation and, where required, a bacterial reverse mutation assay (Ames test [10]). This is certainly useful in clarifying that *in silico* SAR assessments should focus specifically on mutagenicity. Other SAR models for other toxicological endpoints such as chromosomal activity and carcinogenicity exist within many of the *in silico* systems utilized; however these do not need to be specifically applied in the evaluation of impurities; the primary focus is mutagenicity. It can be further concluded that in terms of *in vitro*/*in vivo* assays, no other test other than a reverse mutation assay is required, precluding in particular a mammalian cell assay defined as part of impurity qualification within ICH Q3A [6].

What is less clear is if there is evidence that an impurity is clastogenic/anugenic, whether or not this can be ignored or if limits should be based on an evaluation of the available data. That is, a limit calculated based on no observed effect level (NOEL) using the permitted daily exposure (PDE) calculation shown in ICH Q3C [11].

Also reaffirmed within this section is the fact that the guideline remains, as per earlier regional EMA and FDA guidelines, based on the TTC principle [12–16] and a limit of 1.5 μg/day based on a 1 in 100,000 risk following lifetime exposure (70 years). In reality it is doubtful that this was ever subject to considerable discussion. While clear flaws within the TTC have been identified by Snodin [17] and Delaney [18], what is singularly lacking is any scientifically justified alternative. The TTC is without doubt an overtly conservative interpretation of the risk, one that employs multiple worse-case scenarios, but to

date an alternative to it remains elusive. This innate conservatism is even recognized within the guideline itself:

> The use of a numerical cancer risk value (1 in 100,000) and its translation into risk-based doses (TTC) is a highly hypothetical concept that should not be regarded as a realistic indication of the actual risk. Nevertheless, the TTC concept provides an estimate of safe exposures for any mutagenic compound. However, exceeding the TTC is not necessarily associated with an increased cancer risk given the conservative assumptions employed in the derivation of the TTC value. The most likely increase in cancer incidence is actually much less than 1 in 100,000.

Finally, within this section a passing reference is made to metabolites. The guideline simply states that where an impurity is also a metabolite, its mutagenic potential should be addressed through evaluation of the metabolite. Again this was a specific area highlighted within the concept paper; however it is difficult to see how this has been meaningfully addressed and sits as perhaps the one remaining area of uncertainty following publication of ICH M7. The issue is though examined in detail by Dobo et al. [19].

24.2.4 Considerations for Marketed Products

Section 4 of the guideline is intended to be applied to products that were either marketed prior to or after the adoption of this guideline. The two types of product are treated differently. For those marketed after implementation, a further set of requirements (section 8.5, Life Cycle Management) apply.

The need for further evaluation is defined in terms of changes.

24.2.4.1 Changes to Drug Substance

This states that changes to the DS manufacturing route or process should be evaluated to determine whether they result in any new mutagenic impurities or higher acceptance criteria for existing mutagenic impurities. An important point is that the reevaluation of impurities should focus on those stages impacted by the change and that other stages and their associated impurities unaffected by the change do not require reevaluation.

Another important point is that changes should be assessed from the registered starting material (RSM) onward. However, this should be put into the context of where in the synthesis the RSM is introduced (see ICH Q11 [20]). If the RSM is introduced four/five stages before the active pharmaceutical ingredient (API), then there is likely to be effective purging of any mutagenic impurities in the downstream chemistry, whereas for an RSM introduced in the penultimate stage, such an assessment is very important. This is reflected

in section 5.1 (Relationship between the Risk Assessment and Testing of Starting Materials):

> For starting materials that are introduced late in the synthesis of the drug substance (and where the synthetic route of the starting material is known) the final steps of the starting material synthesis should be evaluated for potential mutagenic impurities.

This may suggest that in the context of an existing product where a starting material is introduced late in the synthesis and changes are subsequently made to the route/process of the material, in such instances, a reevaluation would be necessary.

24.2.4.2 Changes to Drug Product

In the context of changes to the DP, such as a modified formulation, the guideline makes clear that the focus is specifically on the DP and on any new or increased levels of a mutagenic degradant arising as a result of that change. Critically it makes clear that changes to the formulated product do not trigger a reevaluation of the route of synthesis of the DS, unless there is a concomitant change.

24.2.4.3 Changes to Clinical Use

This applies where there is a change in either dose or duration (increase) or a change in indication from a serious or life-threatening condition to an indication for a less serious condition where the existing impurity acceptable intakes (AIs) may no longer be appropriate. An obvious example of the latter would be in instances where an anticancer treatment used in late-stage disease for which limits have been based on ICH S9 [9] is then used for either a revised patient population, for example, adjuvant therapy, or a new non-oncology indication.

Another important point that relates to the innate conservatism of the TTC is the statement that changes to patient population in terms of age do not trigger the need for reassessment and revision of limits. Previously the draft FDA guideline [4] had indicated the need to modify limits for pediatric indications. This has been removed from the ICH M7 guideline.

In addition, the earlier FDA draft guideline [4] had indicated that different limits may be applicable dependent on the planned route of delivery, that is, oral, pulmonary, etc. Similarly this has been removed from the ICH M7 guideline.

24.2.5 Further Considerations

Although unclear from the title, this essentially relates to the situation where a new impurity is discovered. In such instances the guideline makes clear that a structural alert alone is insufficient to trigger a concern; there must be new safety data, that is, evidence of mutagenicity.

Although it may seem an unlikely scenario, there are nevertheless many concerns over how this will be managed in practice. With new information being generated almost daily in research laboratories worldwide, how much due diligence effort should companies expend to ensure that there is no new safety data pertaining to registered impurities? For example, the author is aware of examples where positive Ames data was generated in a research laboratory on a chemical that was coincidentally also a registered impurity on an existing commercial product.

For example, how should authorities be informed? What would be the impact on the current marketing authorization should a new mutagenic impurity be identified? Given the likely long-term presence, would the authorization holder be given a period of time to reduce levels or would the product be suspended?

24.2.6 Drug Substance and Drug Product Impurity Assessment

This section (section 5) is essentially the start point for the overall risk assessment process itself. Critically in practical terms, this essentially advocates the same approach as previous guidelines. The emphasis remains focused on actual impurities and potential impurities *likely* to be present in the API/DP. Another important aspect of this section of the guideline is that it also looks to link the ICH M7 [1] to ICH Q3A [6]/Q3B [7] reporting and identification requirements. Actual impurities are defined as those observed in the DS above the ICH Q3A reporting thresholds. Identification of actual impurities is expected when the levels exceed the identification thresholds outlined by ICH Q3A [6]. This confirms the primacy of ICH Q3A in terms of identification thresholds.

In practical terms this means that there is no requirement to attempt to identify every possible impurity at a TTC level. Such an attempt would be completely impractical. Instead the guideline tacitly accepts the finite but low risk of a mutagenic impurity existing, unidentified, at level below the ICH Q3A identification threshold. This is considered to be an acceptably low risk, provided a comprehensive risk assessment is made of the synthetic process, its related impurities, DP manufacturing process, and associated degradants.

24.2.6.1 Synthetic Impurities

Unlike earlier regional guidelines ICH M7 clearly defines a start point in terms of the risk assessment, indicating that such an assessment may begin at the starting materials. As described earlier there is a caveat that relates to the proximity of the starting material to the final API. The guideline states that for starting materials that are introduced late in the synthesis of the DS (and where the synthetic route of the starting material is known), the final steps of the starting material synthesis should be evaluated for potential mutagenic impurities. One may consider—what is meant by final steps? Two steps or perhaps

three? This is not defined. Despite this uncertainty the guideline provided by ICH M7 is much clearer in terms of its starting point than previously defined and should make the process simpler as a consequence.

Another important aspect of this section of the guideline is the clear recognition that proximity to the final API is a key factor in determining risk (i.e., purging capacity of the downstream process), that early stages will, in general, represent a lower risk of carry-over than later stages. This point is returned to later in the guideline within the control options section and is again a new key aspect of the guideline.

In the context of synthetic impurities, mutagenic impurities can arise via three sources:

1) Mutagenic reagents used deliberately in the synthesis. Many of the common reagents used in the synthesis of the DS are mutagenic. The use of such reagents, that is, methyl iodide, epichlorohydrin, etc., is effectively unavoidable; it is simply impractical to construct C—C or C—N bonds without the use of such reagents [21].
2) Mutagenic intermediates. Often the use of a deliberately formed, highly reactive intermediate is required—examples include tosylates, hydrazides, and epoxides. Such an intermediate is deliberately utilized to effect an efficient synthesis.
3) Side reactions. Perhaps the most difficult to assess are those impurities formed as a result of predictable side reactions. Wherever possible this should be based on existing scientific knowledge. Nowhere is this better illustrated than the case of sulfonate esters. Sulfonate esters are theoretical impurities purported to form through the reaction between sulfonic acids and alcohols. These have long been subject to considerable regulatory scrutiny despite compelling scientific evidence that the risk is negligible and that effective control strategies can be readily implemented [22–24].

24.2.6.2 Degradation Products

Unlike earlier guidelines, ICH M7 specifically addresses the issue of mutagenic degradants. Similar to DS impurities, the guideline makes clear the primacy of ICH Q3B [7] and the identification thresholds for the product. It also makes clear again the need to focus on degradants likely to be present in the final DP. While clearly helpful, this nevertheless does not define how the risk posed by degradants should be evaluated. The guideline provides some advice defining degradants in terms of actual degradants and probable degradants. Actual degradants are those observed to form over prolonged storage at ambient temperature, that is, ICH long-term accelerated storage conditions. Probable impurities are defined in terms of those observed under accelerated conditions, for example, 40°C/75%. Reference is made within the guideline to the use of knowledge derived from stress studies. The evaluation of mutagenic

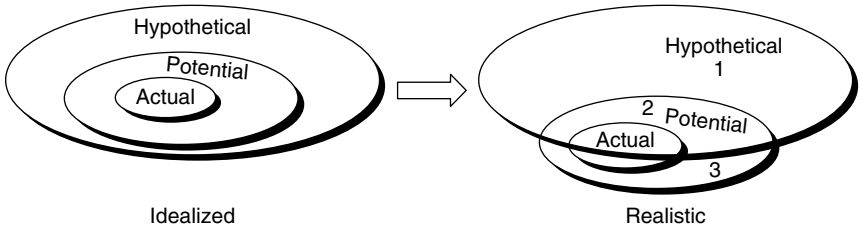


Figure 24.1 Interrelationship between degradant classes.

degradants is examined in detail by Baertschi et al. [25]. The use of stress studies to identify major degradative pathways and their associated primary degradants is underpinned by the established relationship between degradants formed under stress conditions and those seen under ambient conditions. Baertschi et al. [25] demonstrated that degradants formed under ambient conditions were a contained "subset" of those observed to form under stressed conditions; this is illustrated in Figure 24.1, where the relationship between idealized and realistic degradation knowledge landscapes is considered.

Hypothetical degradants arise from *in silico* and *in cerebro* assessments. Potential degradants are observed as major degradation products in stress testing and accelerated stability testing. Actual degradation products are those that arise under ICH long-term (real-time) storage conditions.

Another important consideration in the assessment of degradants is the appropriate identification threshold within the context of a stress study. The identification threshold defined within ICH Q3B [7] for a DP with a dose of >10 mg—2 g is set at 0.2% or 2 mg. This is relative to a typical maximal level of degradation of typically 2% total degradation in DP. In the context of a stress study where degradation levels may well exceed 10% total, an adjustment factor of five to the identification threshold would seem pragmatic, raising this to a value of 1.0%. Using such a structured approach will ensure that impurities identified during a stress study are the primary degradants and hence commensurate with the focus of the guideline on degradants likely to be present in the final DP.

Overall in terms of the impurity assessment, the impurities for consideration are reflected in Figure 24.2.

24.2.7 Hazard Assessment

The emphasis of the guideline (section 6) now shifts and focuses on an assessment of the mutagenic potential of impurities identified in the preceding risk assessment. Such an assessment is typically made through the use of *in silico* SAR systems. The guideline defines the need to apply two (Q)SAR methodologies: one methodology should be expert rule based and the second methodology should be statistical based. However the guidance doesn't define

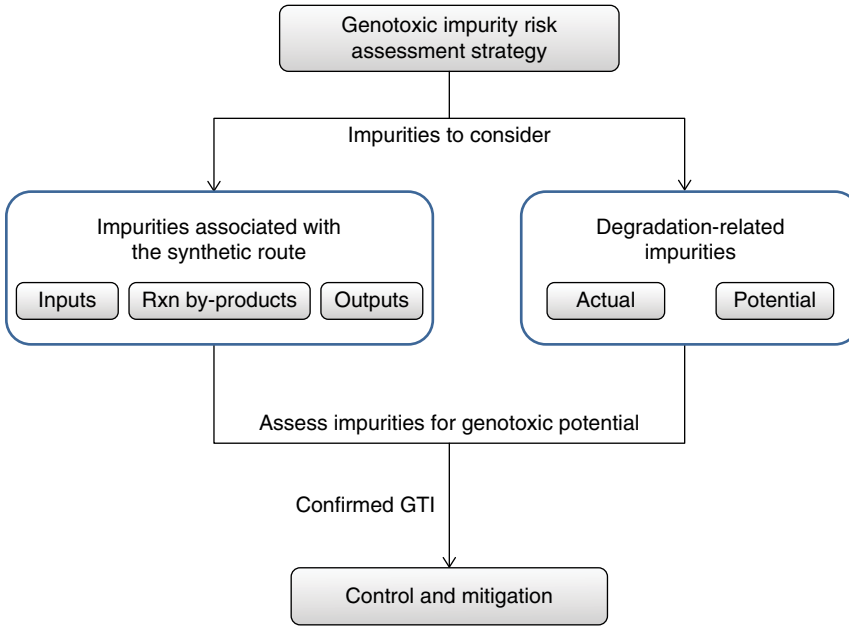


Figure 24.2 Potential sources of mutagenic impurities.

which software packages are preferable; this decision is left to the end user. Importantly it also highlights the need for an expert evaluation of the results. The rationale for the need for two methodologies remains controversial. Two major cross-industry evaluations have concluded that there is little, if any, advantage in using two methodologies over predictions made using simply a rule-based system when predictive results are compared with the outcome of experimental assays [26, 27]. A further perspective was however provided by Powley (FDA) [28].

The use of two methodologies throws up a number of different permutations, including not only situations where predictions are conflictory in nature but also out-of-domain predictions. These arise where the molecule in question, or at least a proportion of the molecule, is not recognized by the training set of the *in silico* tool, and hence it cannot accurately predict its mutagenic potential. In such circumstances expert evaluation is required to make an overall consensus prediction. Barber et al. [29] examine this in detail. They describe the various scenarios potentially encountered (Figure 24.3), examining how to address conflictory predictions as well as out-of-domain predictions. Barber et al. provide advice on how to challenge predictions made by both rule-based and QSAR systems as well as provide a series of examples that serve to provide effective practical illustration of the key points made within the paper.

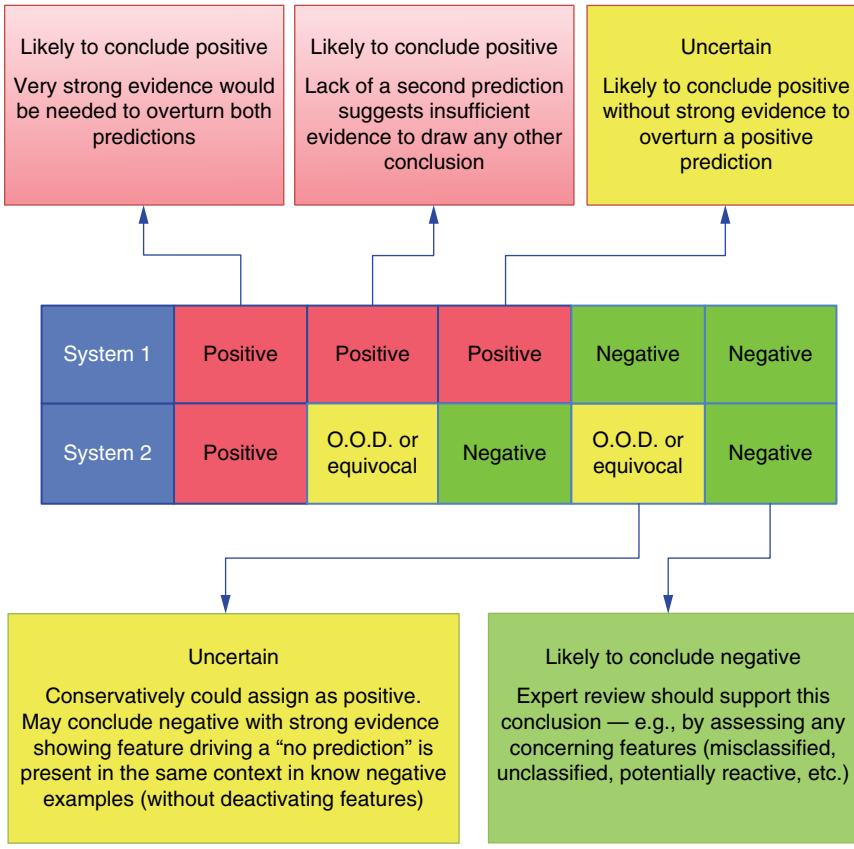

Likely to conclude positive
Very strong evidence would be needed to overturn both predictions
Likely to conclude positive
Lack of a second prediction suggests insufficient evidence to draw any other conclusion
Uncertain
Likely to conclude positive without strong evidence to overturn a positive prediction
System 1
Positive
Positive
Positive
Negative
Negative
System 2
Positive
O.O.D. or equivocal
Negative
O.O.D. or equivocal
Negative
Uncertain
Conservatively could assign as positive. May conclude negative with strong evidence showing feature driving a "no prediction" is present in the same context in know negative examples (without deactivating features)
Likely to conclude negative
Expert review should support this conclusion — e.g., by assessing any concerning features (misclassified, unclassified, potentially reactive, etc.)

Figure 24.3 Decision matrix when evaluating two *in silico* predictions.

In a related study Greene et al. [30] examined the relative predictive performances of popular commercial *in silico* systems. Using a data set of some 801 chemicals and pharmaceutical intermediates, they showed that the overall accuracy of each of the systems was generally comparable, ranging from 68 to 73%; however, their studies showed significant differences in the sensitivity of each system (i.e., how many Ames-positive compounds are correctly identified), results varying between 48 and 68%. The studies did not, however, identify any standout system or specific combination of rule-based/QSAR systems. Perhaps the most significant finding of the studies was the number of contradictory predictions observed when two different methodologies were applied, that is, those where one system predicted positive and the other did not or the statistical models were not able to make a prediction. Over one-third of all the compounds in this 801 compound data set were seen to give a conflictory prediction. The authors concluded that there is clearly a need for expert opinion to be applied to determine the appropriate classification and ensure measures are

Table 24.1 Impurities classification with respect to mutagenic and carcinogenic potential and resulting control actions.

Class	Definition	Proposed action for control (details in sections 7 and 8)
1	Known mutagenic carcinogens	Control at or below compound-specific acceptable limit
2	Known mutagens with unknown carcinogenic potential (bacterial mutagenicity positive, no rodent carcinogenicity data)	Control at or below acceptable limits (appropriate TTC)
3	Alerting structure, unrelated to the structure of the drug substance; no mutagenicity data	Control at or below acceptable limits (appropriate TTC) or conduct bacterial mutagenicity assay: If non-mutagenic = class 5 If mutagenic = class 2
4	Alerting structure, same alert in drug substance or compounds related to the drug substance (e.g., process intermediates) that have been tested and are non-mutagenic	Treat as non-mutagenic impurity
5	No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity	Treat as non-mutagenic impurity

taken to maximize patient safety. This review should consider not only conflictory predictions but also those where negative predictions were made in both systems, including careful consideration of the chemical class.

Ultimately the outcome of any such assessment is then classified using the system defined by Müller et al. [31]. This is shown in Table 24.1.

A particular challenge with respect to class 4 compounds is defining structural similarity. Mathematical approaches such as Tanimoto scores may be utilized; however great care is required in their use and similarity cannot simply be defined by a score exceeding a predefined threshold. In all cases it is important to assess the environment, both steric and electronic, in close proximity to the alerting moiety within the impurity in question.

Based on the outcome of the SAR assessment, for those compounds considered a concern, in particular those classified as class 3, further evaluation in the form of testing may be performed. The earlier scope section of the ICH M7 guideline makes clear that the emphasis is on mutagenic impurities and that the bacterial reverse mutation test (Ames) [10] should be used to follow up any SAR alert.

Table 24.2 Tests to investigate the *in vivo* relevance of *in vitro* mutagens (positive bacterial mutagenicity).

In vivo **test**	**Factors to justify choice of test as fit for purpose**
Transgenic mutation assays	For any bacterial mutagenicity positive. Justify selection of assay tissue/organ
Pig-a assay (blood)	For directly acting mutagens (bacterial mutagenicity positive without S9[a])[b]
Micronucleus test (blood or bone marrow)	For directly acting mutagens (bacterial mutagenicity positive without S9) and compounds known to be clastogenic[b]
Rat liver unscheduled DNA synthesis (UDS) test	In particular for bacterial mutagenicity positive with S9 only
	Responsible liver metabolite known
	To be generated in test species used
	To induce bulky adducts
Comet assay	Justification needed (chemical class-specific mode of action to form alkaline labile sites or single-strand breaks as preceding DNA damage that can potentially lead to mutations)
	Justify selection of assay tissue/organ
Others	With convincing justification

Source: ICH M7(R1) [32]. Courtesy of ICH M7.
[a] S9—supernatant fraction obtained from an organ (usually the liver) homogenate—contains cytosol and microsomes. The microsome component of the S9 fraction contains cytochrome P450 isoforms (phase I metabolism) and other enzyme activities.
[b] For indirect-acting mutagens (requiring metabolic activation), adequate exposure to metabolite(s) should be demonstrated.

In addition this section provides an overview of potential *in vivo* follow-up tests that can be utilized in order to investigate further a positive bacterial assay. The tests themselves are described in detail in Table 24.2 (based on note 3 within the guideline).

The guideline states that such tests can used to assess the *in vivo* relevance of the positive findings of the *in vitro* bacterial reverse mutation test, suggesting that the results may support the establishment of a compound-specific limit. In reality at this present time, there are no practical examples of the use of such data and how it might be used to modulate the outcome of the bacterial assay.

24.2.8 Risk Characterization

This section, section 7 in the guideline, outlines the risk characterization principles used to define acceptable limits for compounds classified in groups 1, 2, or 3 (see Table 24.1).

24.2.8.1 Acceptable Intakes Based on Compound-Specific Risk Assessments

24.2.8.1.1 Mutagenic Impurities with Positive Carcinogenicity Data (Class 1)

It is important to note that the guideline specifically stipulates that where adequate carcinogenicity data exist, it should be used to calculate a compound-specific AI or ADI. It also outlines that the approach should mirror that of the derivation of the TTC itself, that is, linear extrapolation to a risk value of 1 in 100,000 analogous in risk terms to the TTC. It also states that other established risk assessment practices such as those used by international regulatory bodies may be applied to calculate either AIs or the actual values themselves used. This apparently helpful statement does in fact lead to considerable confusion, should, for example, the value for a particular compound specified by the US EPA (or other agencies) be simply adopted or in such instances should the available data be evaluated using the linear extrapolation? The guideline provides no clear statement on such a point, nor does it provide any specific example. In practice it would seem appropriate to use the default approach of linear extrapolation where data are available. Dobo et al. [33] recently reported on various ADIs that can be generated for hydrazine, exemplifying the considerable ambiguity that can be found when trying to generate a compound-specific ADI for regulatory use.

Linked to this section is note 4 where a specific example calculation is provided. The calculation outlines the determination of an AI for ethylene oxide. It is surprising that ethylene oxide was chosen as it is a gas, with good purging potential and of little synthetic utility, making its presence in final product very unlikely. Furthermore there is strong evidence that it is also generated endogenously [34]. In terms of the calculation itself, it is relatively straightforward. TD_{50} values are taken from the Carcinogenicity Potency Database (CPDB) for both rat and mouse, with the more conservative value being selected as 21.3 mg/kg/day (rat) and the limit calculated divided by 50,000 to adjust to a 1 in 100,000 risk and multiplied by the internationally accepted average human body weight (50 kg) to give an ADI of 21.3 μg/day for lifetime exposure.

On the face of it, this looks relatively straightforward; however this is a simple example. In reality this is often far more complex. In many cases data are available for multiple carcinogenicity studies; within the CPDB these are combined and reported in terms of the harmonic mean. The studies involved may be of variable quality, for example, insufficient duration, low animal numbers, limited number of doses studied, etc. Another important factor for consideration is tumor site and relevancy, for example, forestomach tumors in rodents. Such tumors are often associated with local irritation/inflammation and are considered nonrelevant to humans, both from a physiological perspective (humans have no forestomach) and from an exposure perspective; the impurity at the low levels observed within a pharmaceutical product renders it extremely unlikely to result in such irritation.

As a result of the complexity described, a cross-industry initiative was established, which looked to develop an addendum table to the guideline. Included within this would be agreed limits for a range of common mutagenic/carcinogenic reagents. In addition to the actual agreed AIs, specific criteria were established to allow for the calculation of limits for other reagents in addition to those captured in the addendum. This ultimately culminated with the publication of an addendum to the main guideline finalized in June 2017 [32]. The principles underpinning the addendum are examined in the following text.

24.2.8.2 Principles for the Establishment of AIs

The ICH M7 addendum [32] sets out a series of principles.

24.2.8.2.1 Studies

Despite the fact the CPDB includes criteria for inclusion, the quality of studies in the CPDB is nevertheless variable. In the derivation of the addendum table, the following criteria were defined:

- >50 animals per dose per sex
- >3 dose levels
- Concurrent controls
- 7-Day dosing
- Lifetime duration

The more robust studies based on these criteria were used to derive limits.

24.2.8.2.2 Selection of Tumor and Site

Values were calculated from the lowest TD_{50} of a particular organ site for an animal species and sex using data derived from the most robust studies. Rather than simply applying the harmonic mean provided by the CPDB, the lowest TD_{50} was used as it was considered to provide a more conservative estimate. In addition mixed tumor types (e.g., adenomas and carcinomas) in one tissue (e.g., liver) were used where appropriate as this often gives a more sensitive potency estimate.

Another important aspect of the evaluation was assessment of human relevance. An example of such assessment is that of *p*-chloroaniline. In studies of *p*-chloroaniline the most sensitive site for tumor induction was the spleen. However such tumors are associated with hemosiderosis [35]; the mode of action is associated with a nonlinear dose–response, that is, thresholded, and thus considered nonrelevant to humans at the low concentrations associated with a pharmaceutical impurity. Thus for *p*-chloroaniline, liver tumors with a higher TD_{50} were used for the linear extrapolation to calculate the AI.

24.2.8.2.3 Route of Administration

The TTC limit was derived using data from a large range of studies encompassing multiple routes of administration. Thus in the derivation of AIs unless the

tumor sites appeared to be route specific, the TD_{50} from the route with the lower TD_{50} was selected for the AI calculation. If tumors were considered site specific (e.g., inhalation exposure resulting in respiratory tract tumors with no tumors at distal sites) and the TD_{50} was lower than for other routes, then a separate AI was developed for that route.

24.2.8.2.4 Defined Limits

Table 24.3 describes the limits within ICH M7 addendum including the methodology employed in their calculation.

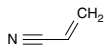
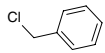
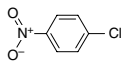
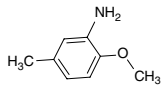
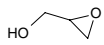
Many of the limits derived are substantively higher than the standard TTC limit. Similar to the TTC itself, such limits may also be adjusted to account for less-than-lifetime dosing. Where this occurs, limits are capped at a level of 500 μg/day.

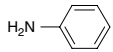
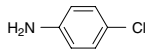
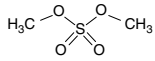
What is also interesting is what is missing from the addendum. One obvious omission is formaldehyde, perhaps surprising given within the step 2 document the inclusion of a specific category of endogenous and food exposure. Exogenous and endogenous exposure to formaldehyde is on a vast scale in comparison with any exposure likely from an impurity perspective. Formaldehyde exposure occurs in air, water, and food, and formaldehyde is a by-product of endogenous metabolism. The mean outdoor concentrations range from 2.5 to 15.7 $\mu g/m^3$ [36]. It is found in ozonized drinking water at concentrations up to 30 μg/l [37]. It is a common endogenous component of biological materials and is a naturally occurring component of many foods such as meat, dairy products, fruit, and vegetables. Levels of daily exposure to formaldehyde via the dietary route have been estimated in the range of 1.5–14 mg/day [35]. Formaldehyde is also a product of normal human metabolism and is essential for the biosynthesis of certain amino acids. The human body produces and uses ~50 g of formaldehyde a day that is rapidly metabolized and cleared from blood [38, 39]. Indeed a route-specific limit was developed for inhaled formaldehyde by Conolly et al. [40], an AI for inhaled formaldehyde of 5 mg/day being proposed.

24.2.8.2.5 Other Approaches

In addition the guideline also outlines the potential to utilize "surrogate data," that is, data from a closely related compound or group of compounds to derive a limit for an impurity where there is no available carcinogenicity data. An example of such a limit is provided via note 5. This outlines the derivation of a class-based limit for monofunctional alkyl chlorides. The evaluation, originally conducted by Brigo et al. [41] proposes a class-based limit 10 times higher than the standard TTC based on the low carcinogenic potency of such compounds, values ranging from 36 to 1810 mg/kg/day ($n = 15$). It is interesting to note that in early drafts of the ICH M7 guideline note 5 referred to monofunctional alkyl halides as opposed to simply alkyl chlorides, this "refinement" would seem

Table 24.3 Acceptable intakes (AIs) or permissible daily exposures (PDEs) for mutagenic carcinogens.

Compound	CAS#	Chemical structure	AI or PDE (µg/day)	Comment
Linear extrapolation from TD_{50}				
Acrylonitrile	107-13-1		6	TD_{50} linear extrapolation
Benzyl chloride	100-44-7		41	TD_{50} linear extrapolation
Bis(chloromethyl) ether	542-88-1		0.004	Unusually potent carcinogen (below TTC)
1-Chloro-4-nitrobenzene	100-00-05		117	PDE based on threshold mode of action (hemosiderosis)
p-Cresidine	120-71-8		45	TD_{50} linear extrapolation
Dimethylcarbamoyl chloride	79-44-7		5 (oral) 0.6 (inhalation)	TD_{50} linear extrapolation
Ethyl chloride	75-00-3		1810	TD_{50} linear extrapolation
Glycidol	556-52-5		4	TD_{50} linear extrapolation

Hydrazine	302-01-2	$H_2N—NH_2$	42 (oral) 0.2 (inhalation)	TD_{50} linear extrapolation
Methyl chloride	74-84-3	$Cl—CH_3$	1360	Defaulted to TD_{50} linear extrapolation even though tumors were likely species specific
Threshold-based PDE				
Aniline	62-53-5		720	PDE based on threshold mode of action (hemosiderosis) for spleen
Hydrogen peroxide	7722-84-1	HO — OH	6960	PDE based on threshold (oxidant stress where protective antioxidant mechanisms overwhelmed)
Hydroxylamine	7803-49-8	$HO—NH_2$	2	PDE based on threshold mode of action (hemosiderosis) for spleen
Endogenous and food exposure				
For future compounds				
Other cases				
p-Chloroaniline *p*-Chloroaniline HCl	106-47-8 20265-96-7		34	AI based on liver tumors for which mutagenic mode of action cannot be ruled out (not most sensitive site, which was spleen tumors associated with hemosiderosis)
Dimethyl sulfate	77-78-1		1.5	Carcinogenicity data available, but inadequate to derive AI. Defaulted to TTC

Source: ICH M7(R1) [32]. Courtesy of ICH M7.

Table 24.4 Acceptable intakes for alkyl bromides.

			TD_{50} (mg/kg/day)	
CAS	**Compound name**	**Ames**	**Rat**	**Mouse**
26148-68-5	Methyl bromide	Positive		49.8[m]
75-25-2	Tribromomethane	Positive	648[m]	Negative
74-96-4	Ethyl bromide	Positive	149[n]	535
106-93-4	1,2-Dibromoethane	Positive	1.52[m]	7.45[m,v]
540-51-2	Bromoethanol	Positive		76.1[m]
3296-90-0	2,2-Bis(bromomethyl)-1,3-propanediol	Positive	111[m]	137[m]
36711-31-6	Bis(2,3-dibromopropyl)phosphate		32[m]	NA
126-72-7	Tris(2,3-dibromopropyl)phosphate	Positive	3.83[m]	128[m]
488-41-5	Dibromomannitol	Positive	27.6[i,m]	14.9[i,m]
10318-26-0	Dibromodulcitol	Positive	8.37[i]	11[i,m]
593-60-2	Vinyl bromide	Positive	18.5[m]	
17157-48-1	Bromoacetaldehyde			Negative
105-36-2	Ethyl bromoacetate	Positive		Negative

unnecessary as, although there are less data available for monofunctional alkyl bromides, the available data, as shown in Table 24.4, suggest that a similar value would be appropriate (see data for methyl bromide, ethyl bromide, bromoethanol).

24.2.8.2.6 Mutagenic Impurities with Evidence for a Practical Threshold

There is increasing evidence that even for mutagenic carcinogens, there is a discernible threshold [42, 43]. Where this can be substantiated, the guideline advocates that rather than calculating limits through linear extrapolation of TD_{50} values, AIs for such compounds can be based on the identification of a NOEL and use of uncertainty factors (see ICH Q3C [11]) to calculate a PDE when data are available. The crucial term is *when data are available*. At present there are no defined criteria as to the data required to establish a threshold for a mutagenic carcinogen. In the aftermath to the Viracept incident [44], Gocke et al. [45] conducted exhaustive studies to examine the patient risk associated with exposure to a mutagenic carcinogen, ethyl methane sulfonic acid (EMS). Two studies examining potential DNA damage were performed in mice; chromosomal damage was assessed using a micronucleus assay, and gene mutations were detected using the MutaMouse™ transgenic model. In addition, experiments designed to extrapolate animal exposure to humans were

undertaken. Studies for chromosomal damage and mutations in mice demonstrated a clear threshold effect with a NOEL for EMS at 25 mg/kg/day under chronic dosing conditions being established. Exposure analysis (Cmax) demonstrated that ~370-fold higher levels of EMS than that ingested by patients were needed to saturate known, highly conserved, error-free, mammalian DNA repair mechanisms for alkylation. Despite this definitive evidence of a clear threshold controversy remains as to whether or not the derived AI of 100 μg/day [45] would be accepted. In the absence of such definitive guidance, combined with the extensive studies required to demonstrate a threshold, it is unlikely that such approaches will be used extensively, certainly not at this precise point in time.

Extensive research is though being undertaken in this area, examining in particular methodology for determination of the point of departure for *in vitro* and *in vivo* studies [46–48].

24.2.8.3 Acceptable Intakes for Class 2 and Class 3 Compounds

In the absence of specific carcinogenicity data, the ICH M7 guideline outlines AIs based on the TTC. Critically, for both products in clinical development and marketed products, limits are based on duration of exposure, that is, a staged approach [31]. The recognition that limits should be based on duration of exposure irrespective of phase of development or marketing status is a major step forward as prior to finalization of the guideline; the lifetime limit of 1.5 μg/day was routinely applied to marketed products, irrespective of their intended use and likely duration.

The calculation of less-than-lifetime AIs is ultimately based on the principle of Haber's law, where concentration (C) × time (T) = a constant (k). The limits are described in Table 24.5.

Within this section of the guideline is a specific section dedicated to clinical development. This proposes that for studies <14 days in duration, limits only apply to those impurities for which there is actual *in vitro* safety data (class 2) and no specific controls are required over levels of impurities where there is only a structural alert (class 3). While this is an interesting concept that would appear to provide flexibility, it remains to be seen whether or not this is utilized to a significant extent by applicants. In many cases it is unlikely that material will be restricted to use in clinical studies of <14 days' duration, in which case, in order to utilize material for longer-duration studies, compliance with the limits outlined in Table 24.5 will be required for both class 2 and 3 impurities.

In the context of marketed products, a particular challenge is defining duration. Again this is addressed through a specific note in the guideline (note 7), taking the form of a table where, based on specific therapeutic areas, the likely treatment durations are discussed. Useful advice is provided within the guideline itself where it is made clear that the duration of use should be defined based on typical use. This recognizes that, in some cases, a subset of the

Table 24.5 Acceptable intakes for an individual impurity.

Duration of treatment	≤1 month	>1–12 months	>1–10 years	>10 years to lifetime
Daily intake (μg/day)	120	20	10	1.5

Source: ICH M7(R1) [32]. Courtesy of ICH M7.

Table 24.6 Acceptable total daily intakes for multiple impurities.

Duration of treatment	≤1 month	>1–12 months	>1–10 years	>10 years to lifetime
Total daily intake (μg/day)	120	60	30	5

Source: ICH M7(R1) [32]. Courtesy of ICH M7.

population of patients may extend treatment beyond the marketed products' anticipated duration of use. It states that in such instances, the increase in risk is negligible.

24.2.8.4 Multiple Impurities

One of the questions posed within the original ICH M7 concept paper relates to potential synergistic effect of multiple mutagenic impurities, especially where they are closely related. Based on the work of Bercu et al. [49], the guideline concludes that such effects are unlikely at the low levels potentially present and hence permits the presence of up to three mutagenic impurities to give a total AI as defined in Table 24.6.

It is important to note that this total only applies to specified class 2 and 3 impurities. In reality most synthetic routes will utilize multiple mutagenic reagents/intermediates; however due to the innate reactivity of the reagents in question and the downstream process conditions, few actually pose a substantive risk in terms of potential carry-over. This is discussed in detail in the control section. The following example, as shown in Figure 24.4, seeks to illustrate the substantive difference in risk between two alkyl halides used in this synthesis.

The relative risks associated with the two alkylating agents can be assessed. Allyl bromide is highly reactive and it is very likely that it will be consumed in the step where it is deliberately introduced (step 1). Further purging is likely to occur in the downstream process culminating in stage 3 where bromopropylamine is introduced, where, if still present in residual amounts, allyl bromide will compete with bromopropylamine to react with the intermediate alcohol, potentially yielding the diallyl impurity illustrated. In such examples it is very unlikely that both alkyl bromides would be specified on the DS specification and control would focus on bromopropylamine due to close proximity to the final product.

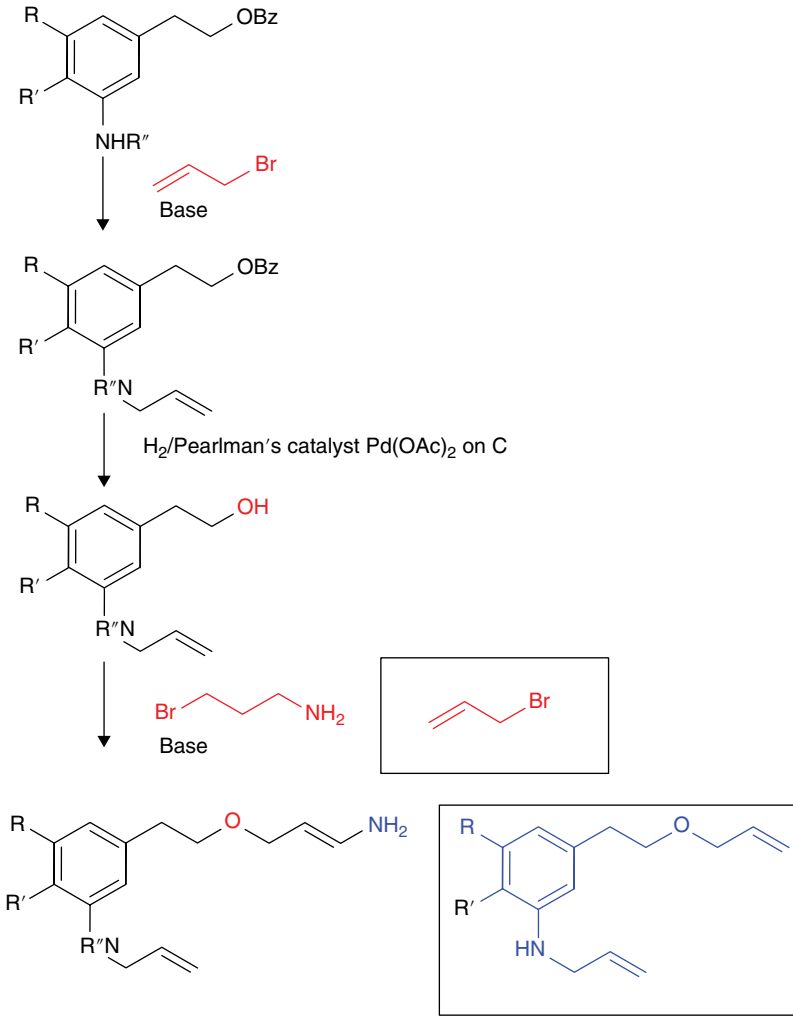


Figure 24.4 Synthetic route utilizing allyl bromide and bromopropylamine.

The total limits described in Table 24.6 also don't apply to class 1 compounds that are controlled based on compound-specific limits. Degradants are also excluded.

24.2.8.5 Exceptions and Flexibility in Approaches

The final area covered in section 7 of the guideline addresses various further scenarios where modified limits may apply. Areas include:

1) Where substantive exposure to the impurity may occur either exogenously or endogenously. Perhaps the clearest example of this is formaldehyde.

2) Exceptions to the use of the appropriate AI can be justified in cases of severe disease, reduced life expectancy, or late-onset but chronic disease or with limited therapeutic alternatives. In practice this is likely to be applicable where effective control has not been possible; in such instances levels will likely be controlled to as low as reasonably practicable (ALARP) and justified following subsequent consultation with regulatory authorities.
3) Where the mutagenic impurity, based on its structure, is considered to likely be a potent carcinogen (cohort of concern), that is, aflatoxin-like, *N*-nitroso, and alkyl azoxy structures, the guideline states that if these compounds are found as impurities in pharmaceuticals, AIs would likely be significantly lower than the AIs defined in this guideline. No specific value is proposed—in principle the presence of such structures is unlikely given that none of the classes described are considered to be of value in terms of utility as synthetic reagents [21].

24.2.9 Control Strategy

One of the most significant aspects of ICH M7 is the control section (section 8). This provides far greater flexibility in terms of strategies to demonstrate absence than are available in the preceding regional guidelines. These are expressed in terms of a series of control options that provide the ability to more widely use chemical-/process-based arguments to assess purging and effectively align the guideline with the risk-based approaches outlined in ICH Q9 [50]. There are four options:

1) Option 1—Include a test for the impurity in the DS specification with an acceptance criterion at or below the acceptable limit.
2) Option 2—Include a test for the impurity in the specification for an input raw material, starting material or intermediate, or as an in-process control, with an acceptance criterion at or below the acceptable limit.
3) Option 3—Include a test for the impurity in the specification for a raw material, starting material or intermediate, or as an in-process control, with an acceptance criterion above the acceptable limit of the impurity in the DS. Such a limit is justified based on clear understanding of the fate and purge in question within the downstream process negating the need for any additional testing later in the process.

   Such an option can be justified when the level of the impurity in the DS will be <30% of the acceptable limit by review of data derived from laboratory-scale spiking experiments.
4) Option 4—Process Control. The highly reactive nature of most mutagenic impurities is such that they are unlikely to survive typical downstream processes at levels of concern in the subsequent DS. To assess the potential carry-over of such impurities requires an understanding of the effect that process conditions have on residual impurity levels. Where there is

sufficient confidence that the level of the impurity in the drug substance will be below the acceptable limit, then no analytical testing may be required and the impurity does not have to be specified.

Examining these options in more detail, many would conclude that the ordering should be reversed. As stated earlier, most mutagenic reagents are highly reactive by nature or deliberate design. They are used in the construct of the drug substance to form the molecular skeleton of the compound. This high reactivity is the reason why they are also effectively purged. The challenge has been to be able to assess the level of the risk without resorting to extensive analytical testing. Such an approach was developed by Teasdale et al. [51, 52]. In this approach calculations are based on an evaluation of the specific physicochemical properties of the impurity in question (e.g., reactivity, solubility, etc.) relative to the downstream processing conditions they will be exposed to. This is based on the scoring system described in Table 24.7.

Table 24.7 Purge values.

Physicochemical parameters	Purge factor
Reactivity	Highly reactive = 100
	Moderately reactive = 10
	Low reactivity/unreactive = 1
Solubility[a]	Freely soluble = 10
	Moderately soluble = 3
	Sparingly soluble = 1
Volatility	Boiling point >20°C *below* that of the reaction/process solvent = 10
	Boiling point ±10°C that of the reaction/process solvent = 3
	Boiling point >20°C *above* that of the reaction/process solvent = 1
Ionizability	Ionization potential of GI significantly different to that of the desired product[b]
Physical processes: chromatography	Chromatography: GI elutes prior to desired product = 100
	Chromatography: GI elutes after desired product = 10
	Others evaluated on an individual basis

[a] This relates to solubility within the context of a recrystallization/isolation process whereby the impurity in question, if highly soluble, will remain within mother liquors and hence be purged from the desired product.

[b] This relates to a deliberate attempt to partition the desired product/GI between an aqueous and organic layer, typically achieved through the manipulation of pH to change the ionized/unionized state of one of the components.

Case Study 3 Reproduced as follows, is concerning:

The step 1 intermediate of a 5-step synthesis is a nitroaromatic compound that may contain low levels of impurity C, a positional isomer of the step 1 intermediate and also a nitroaromatic compound. The amount of impurity C in the step 1 intermediate has not been detected by ordinary analytical methods, but it may be present at lower levels. The step 1 intermediate is positive in the bacterial mutagenicity assay. The step 2 hydrogenation reaction results in a 99% conversion of the step 1 intermediate to the corresponding aromatic amine. This is confirmed via in-process testing. An assessment of purge of the remaining step 1 nitroaromatic intermediate was conducted and a high purge factor was predicted based on purge points in the subsequent step 3 and 4 processing steps. Purge across the step 5 processing step is not expected and a specification for the step 1 intermediate at the TTC-based limit was established at the step 4 intermediate (option 2 control approach). The positional isomer impurity C would be expected to purge via the same purge points as the step 1 intermediate and therefore will always be much lower than the step 1 intermediate itself, and therefore no testing is required and an option 4 control strategy for impurity C can be supported without the need for any additional laboratory- or pilot-scale data.

This specific approach is referenced directly within the guideline.

Examples of the options described are further examined within the guideline through a series of case studies. There are four cases in total: case studies 1 and 2 examine the use of option 3; case study 3, options 2 and 4 combined; and case study 4, the use of option 4.

In case study 3, reproduced above during step 1, nitroaromatic is reported to be converted at high yield (99%) conversion to an aromatic amine; further reductions in level are reported to occur at steps 3 and 4. Certainly where such a scenario encountered in reality, it would seem logical to conduct a risk assessment based on an evaluation of the purging capacity of the process before simply defaulting to a control strategy based on option 2.

24.2.9.1 Considerations for Control Approaches

Within the guideline, this subsection of the overall control strategy section provides further clarification as to what is required to support the specific control options in terms of data. Of particular note is the need to take into consideration the effect of scale, that is, it is important to address the expected scale dependence or independence of any data especially where supporting data is derived from lab- or pilot-scale manufacture.

If options 3 and 4 cannot be justified, then a test for the impurity on the specification for a raw material, starting material or intermediate, or as an

in-process control (option 2) or DS (option 1) at the acceptable limit should be included.

Another important point made within this specific section relates to impurities introduced in the last synthetic step, where it states that an option 1 control approach would be expected unless otherwise justified. The caveat that other options may be justified is an important caveat and potentially allows other control options to be justified based on a scientific risk assessment as opposed to simplistic dogma.

Earlier regional guidelines had been predicated on the application of "ALARP" even where levels were below the TTC. They also talked about avoidance of the use of mutagenic reagents. ICH M7 addresses both points directly. It is not necessary, if the level of the mutagenic impurity is below acceptable limits, to implement limits based on the ALARP principle. Similarly, it is not necessary to demonstrate that alternate routes of synthesis have been explored.

The ALARP principle is applicable in cases where efforts to reduce the level of a mutagenic impurity to below the acceptable limit have proven unsuccessful. In this instance, where provided levels are as low as reasonably practicable, a higher limit may be justified based on a risk/benefit analysis.

24.2.9.2 Considerations for Periodic Testing

This section seeks to provide guideline in terms of the use of periodic testing; however it unfortunately lacks real clarity, failing to provide any definite advice as to when such options are to be applied. A specific concern in the absence of real clarity is that such an approach may be requested even when the control process has been fully justified.

24.2.9.3 Control of Degradation Products

Appearing somewhat out of place the control strategy section also includes specific advice relating to the control of degradation products. The guideline states the need to understand the degradation pathway for a mutagenic degradant and its relevance to the DS and DP manufacturing processes and/or their proposed packaging and storage conditions. It also articulates how either accelerated stability studies (e.g., 40°C/75% relative humidity, 6 months) or kinetically equivalent shorter-term stability studies at higher temperatures in the proposed commercial package may be used to determine the relevance of the degradation pathway prior to initiating longer-term stability studies. This of course makes perfect sense, there being a considerable risk in relying on long-term studies to provide the required assurance of control. Finding out control is inadequate at a late stage, that is, at the end of long-term stability studies, is likely to have a serious impact. Earlier identification of any potential issue would certainly allow more time to consider formulation/packaging options for control. Again, as with synthetic impurities, if formulation development and packaging design options are unable to control mutagenic

degradation product levels to less than the acceptable limit, it may be possible to justify a higher limit based on a risk/benefit analysis.

24.2.10 Life Cycle Management

This section specifically applies to those products approved after the issuance of this guideline. A considerable portion of the section is taken up by what amounts to a description of life cycle management and the need for an appropriate quality system. It is perhaps not wholly clear as to why this is included as it is not specific to the control of mutagenic impurities.

What is clear and useful is the statement that any proposed change to the manufacturing process should be evaluated for the impact on the quality of DS and DP. This evaluation should be based on the understanding of the manufacturing process and should determine if appropriate testing to analyze the impact of the proposed changes is required.

It also stresses the need throughout the life cycle of the product to reassess if testing is recommended when intended or unintended changes occur in the process, highlighting the need to consider this in particular where there is no routine monitoring (option 3 or option 4).

Mention is also made as to the potential use of statistical process control and trending of process measurements, but in the absence of any clear examples, it is not entirely clear as to how this might be utilized.

24.2.11 Documentation

The final section, section 9, of the guideline seeks to provide guidance as to the level of information to include within regulatory submissions pertaining to the assessment and control process for mutagenic impurities. This is split into two sections.

24.2.11.1 Clinical Trail Applications

This is further differentiated based on phase:

1) For phase 1 studies of 14 days or less, the guidance defines the need to provide a description of efforts to mitigate risks of mutagenic impurities focused on class 1 and class 2 impurities and those in the cohort of concern.
2) For phase 1 clinical trials >14 days and for phase 2a clinical trials, it states that in addition class 3 impurities that require analytical controls should be included.
3) For phase 2b and phase 3 clinical development trials, a list of the impurities assessed by (Q)SAR now should be included, as well as a description of the systems used. Any class 1, 2, or 3 actual and potential impurities should be described along with details as to how they are controlled. The results of bacterial mutagenicity tests should also be reported.

It is also stated that chemistry arguments may be used in lieu of analytical data for potential impurities that present a low likelihood of being present, this represents in affect option 4 control. It is assumed therefore that this is applicable irrespective of the phase, despite this not being specifically stated.

24.2.11.2 Common Technical Document (Marketing Application)

The guideline stipulates the following:

1) The mutagenic impurity classification and rationale for this classification for actual and potential process-related impurities and degradation products needs to be provided. Particular emphasis is placed in the guideline on the need to justify the classification of impurities into class 4 and 5 impurities.
2) When bacterial mutagenicity assays were performed on impurities, study reports should be provided for bacterial mutagenicity assays on impurities. What is not clear is whether or not this should be included directly in the submission in the relevant section (would this be within the preclinical section or S3.2 impurities?) of the dossier or as a supplementary reference.
3) Justification for the overall control strategy and any proposed specification. For option 3 and option 4 control approaches, a summary of purge factors and identification of factors providing control (e.g., process steps, solubility in wash solutions, etc.) needs to be provided.

Within both later-stage clinical applications and the CTD, there is an explicit need to properly articulate and justify the classification of impurities based on SAR and any subsequent bacterial mutagenicity data. One potential option would be to include a summary table.

24.2.12 Other Aspects

24.2.12.1 Relationship between ICH M7 and ICH Q3A

Ahead of development of the guideline, the question as to the relationship between guidelines pertaining to mutagenic impurities and ICH Q3A was a considerable concern. These concerns centered on the issue of impurity qualification. For a marketed product any impurity present at level >0.15% requires qualification. In addition to general toxicological qualification, such an impurity requires qualification in terms of its genotoxic potential. It is of course obvious that ICH Q3A predates ICH M7 and indeed earlier regional guidelines; however the question of genotoxic qualification remains. Of particular concern within this was not only does ICH Q3A ask that consideration be given to conduct of assessment of mutagenic potential but also advises that an assessment of chromosomal activity be also considered.

Note 1 within ICH M7 addresses this specific concern. It states that no further qualification for mutagenic potential is required, provided that a SAR assessment of mutagenic potential has been performed on any impurity

present at levels either above or below those defined in ICH Q3A/Q3B. This includes the initial use of (Q)SAR tools to predict bacterial mutagenicity. Only in cases where the amount of the impurity exceeds 1 mg daily dose for chronic administration is further evaluation recommended.

24.3 Conclusions

At the concept stage of the ICH M7 guideline, a series of critical areas were identified, including:

1) Use of *in silico* SAR tools for the assessment of mutagenic potential.
2) What are acceptable levels of genotoxic impurities during drug development?
3) What are acceptable levels of genotoxic impurities for marketing?
4) Should those impurities be regulated differently that are likely to have threshold effects?
5) Should levels of genotoxic impurities be regulated using a TTC approach?
6) Structurally related genotoxic impurities are likely to have similar mechanisms of action. Should these be summed in calculating a TTC?
7) What process of qualification testing should be followed for impurities that are metabolites?
8) What additional data are needed to support having no special restrictions, or a higher ADI than the TTC, for a genotoxic impurity?

Does ICH M7adequately address these points? Yes. Certainly most, if not all, the ambiguity present in earlier guidelines is addressed. Some areas of ambiguity do though remain, certainly management of mutagenic metabolites remains an area of uncertainty, and some new areas added within the guideline may also prove challenging to interpret; this includes periodic testing and some of the documentation requirements. Overall though this is a valuable guideline and one that provides clear and concise guidance as to how to effectively manage MIs.

References

1 ICH M7. Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M7/M7_Step_4.pdf. Accessed on April 10, 2017.
2 Committee for Medicinal Products for Human Use (CHMP). Guidelines on the Limits of Genotoxic Impurities, London, June 26, 2006. CPMP/SWP/5199/02.

3 CHMP Safety Working Party (SWP). Question and Answers on the CHMP Guideline on the Limits of Genotoxic Impurities, London, December 17, 2009. EMEA/CHMP/QWP/251344/2006Q&A.
4 Food and Drug Administration. Guidance for Industry Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches Draft, Center for Drug Evaluation and Research (CDER), December 2008.
5 Ogilvie R., Teasdale A. (2011). Historical Overview of the Development of Genotoxic Impurities Guidelines and Their Impact. In: Teasdale A. (Ed.), *Genotoxic Impurities: Strategies for Identification and Control*, John Wiley & Sons, Inc., Hoboken.
6 ICH (2002). International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Topic Q3A(R). Impurities Testing Guideline: Impurities in New Drug Products (Revision). CPMP/ICH/2738/99: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3A_R2/Step4/Q3A_R2__Guideline.pdf. Accessed on April 10, 2017.
7 ICH (2002). International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Topic Q3B(R). Impurities Testing Guideline: Impurities in New Drug Substances (Revision). CPMP/ICH/2737/99: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3B_R2/Step4/Q3B_R2__Guideline.pdf. Accessed April 10, 2017.
8 Final Concept Paper M7. Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, November 27, 2009. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M7/M7_Final_Concept_Paper_June_2010.pdf. Accessed April 10, 2017.
9 ICH S9. Nonclinical Evaluation for Anticancer Pharmaceuticals, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S9/Step4/S9_Step4_Guideline.pdf. Accessed on April 2017.
10 Ames B.N., Durston W.E., Yamasaki E.E., Lee F.D. *Proc. Natl. Acad. Sci. U. S. A.*, 1973, 72, 2423–2427.
11 ICH Q3C (R5). Guideline for Residual Solvents, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3C/Step4/Q3C_R5_Step4.pdf. Accessed on April 2017.
12 Munro I.C., Ford R.A., Kennepohl E., Sprenger J.G. *Food Chem. Toxicol.*, 1996, 34, 829–867.
13 Munro I.C., Kennepohl E., Kroes R. *Food Chem. Toxicol.*, 1999, 37(2–3), 207–232.
14 Kroes K., Kozianowski G. *Toxicol. Lett.*, 2002, 127, 43–46.
15 Kroes R., Renwick A.G., Cheeseman M., et al. *Food Chem. Toxicol.*, 2004, 42, 65–83.

16 Cheeseman M.A., Machuga E.J., Bailey A.B. *Food Chem. Toxicol.*, 1999, 37, 387–412.
17 Snodin D.J., McCrossen S.D. *Regul. Toxicol. Pharmacol.*, 2013, 67(2), 299–316.
18 Delaney E.J. *Regul. Toxicol. Pharmacol.*, 2007, 49, 107–124.
19 Dobo K., Walker D., Teasdale A. (2011). Human Genotoxic Metabolites: Identification and Risk. In: Teasdale A. (Ed.), *Genotoxic Impurities: Strategies for Identification and Control*, John Wiley & Sons, Inc., Hoboken.
20 ICH Q11. Development and Manufacture Of Drug Substances (Chemical Entities and Biotechnological/Biological Entities), http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q11/Q11_Step_4.pdf. Accessed on April 2017.
21 Elder D., Teasdale A. *Org. Process Res. Dev.*, 2015, 19(11), 1437–1446.
22 Teasdale A., Eyley S.C., Delaney E., et al. *Org. Process Res. Dev.*, 2009, 13, 429–433.
23 Teasdale A, Delaney E., Eyley S.C., et al. *Org. Process Res. Dev.*, 2010, 14, 999–1007.
24 Snodin D, Teasdale A. *Org. Process Res. Dev.*, 2015, 19, 1465–1485.
25 Kleinman M., Baertschi S., Teasdale A., et al. *Org. Process Res. Dev.*, 2015, 19(11), 1447–1457.
26 Dobo K., Greene N., Fred C., et al. *Regul. Toxicol. Pharmacol.*, 2012, 62, 449–455.
27 Sutter A., Amberg A., Boyer S., et al. *Regul. Toxicol. Pharmacol.*, 2013, 67, 39–52.
28 Powley M. *Regul. Toxicol. Pharmacol.*, 2015, 71(2), 295–300.
29 Barber C., Amberg A., Custer L. *Regul. Toxicol. Pharmacol.*, 2015, 73(1), 367–377.
30 Greene N., Dobo K., Kenyon M., et al. *Regul. Toxicol. Pharmacol.*, 2015, 72(2), 335–349.
31 Müller L., Mauthe R.J., Riley C.M., et al. *Regul. Toxicol. Pharmacol.*, 2006, 44, 198–211.
32 ICH M7(R1). Addendum: Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M7/M7_Addendum_Step_2.pdf. Accessed on April 10, 2017.
33 Ellis P., Kenyon M., Dobo K. *Regul. Toxicol. Pharmacol.*, 2013, 65(2), 201–213.
34 Bolt H. *Biochem. Pharmacol.*, 1996, 52, 1–5.
35 Dhareshwar S.S., Stella V.J. *J. Pharm. Sci.*, 2008, 97(10), 4184–4193.
36 Organisation for Economic Co-operation and Development (OECD) (2002) SIDS Initial Assessment Report. Formaldehyde.
37 World Health Organization (WHO) (2005) Background Document for Development of WHO Guidelines for Drinking-Water Quality. WHO/SDE/WSH/05.08/48.

38 Stayner L.T., Elliot L., Blade L., et al. *Am. J. Ind. Med.*, 1988, 13(6), 667–682.

39 Heck H., Casanova-Schmitz M., Dodd P.B., et al. *Am. Ind. Hyg. Assoc. J.*, 1985, 46, 1–3.

40 Conolly R.B., Kimbell J.S., Janszen D., et al. *Toxicol. Sci.*, 2004, 82(1), 279–96.

41 Brigo A., Muller L. (2011). Development of the Threshold of Toxicological Concern Concept (TTC) and its Relationship to Duration of Exposure. In: Teasdale A. (Ed.), *Genotoxic Impurities: Strategies for Identification and Control*, John Wiley & Sons, Inc., Hoboken.

42 Doak S., Johnson G., Quick E., et al. *Cancer Res.*, 2007, 67, 3904–3911.

43 Bailey G.S., Reddy A.P., Pereira C.B., et al. *Chem. Res. Toxicol.*, 2009, 22, 1264–1276.

44 Gerber C., Toelle H.-G. *Toxicol. Lett.*, 2009, 190, 248–253.

45 Gocke E., Ballantyne M., Whitwell M., et al. *Toxicol. Lett.*, 2009, 190, 286–297.

46 Johnson G.E., Soeteman-Hernández L.G., Gollapudi B.B., et al. *Environ. Mol. Mutagen.*, 2014, 55, 609–623.

47 MacGregor J.T., Frötschlb R., White P.A., et al. *Mutat. Res.*, 2015, 783, 66–78

48 MacGregor J.R., Frötschlb R., White P.A., et al. *Mutat. Res.*, 2015, 783, 55–65.

49 Bercu J., Hoffman W., Lee C., et al. *Regul. Toxicol. Pharmacol.*, 2008, 51(3), 270–277.

50 ICH Q9. Quality Risk Management, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q9/Step4/Q9_Guideline.pdf. Accessed on April 10, 2017.

51 Teasdale A., Fenner S., Ray A., et al. *Org. Process Res. Dev.*, 2010, 14(4), 943–945.

52 Teasdale A., Elder D., Chang S.J., et al. *Org. Process Res. Dev.*, 2013, 17(2), 221–230.

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